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ENZYME ADDITION TO THE ANAEROBIC DIGESTION OF MUNICIPAL WASTEWATER PRIMARY SLUDGE

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16. ABSTRACT

This study evaluates the effects of enzyme augmentation on municipal wastewater (MWW) sludge anaerobic digestion. The primary objective was to examine the impact of using enzymes to enhance the degradation of the cellulosic and the oil- and grease-rich sludge fractions. The addition of cellulase enzymes to enhance the anaerobic digestion of the cellulosic component and the addition of lipase enzymes to degrade oil and grease was evaluated in a series of laboratory-scale studies.

The results of this program show that cellulase enzyme augmentation can produce a significant enhancement of the anaerobic digestion of municipal wastewater sludge. The largest effect noted, however, results from the use of a two-phase digestion process to accommodate enzyme addition. Under the conditions studied lipase enzyme has only a minimal effect on anaerobic digestion.

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FOREWORD

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the Nation's land, air, and water systems. Under a mandate of national environmental laws, the agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. The Clean Water Act, the Safe Drinking Water Act, and the Toxic Substances Control Act are three of the major congressional laws that provide the framework for restoring and maintaining the integrity of our Nation's water, for preserving and enhancing the water we drink, and for protecting the environment from toxic substances. These laws direct EPA to perform research to define our environmental problems, measure the impacts, and search for solutions.

The Water Engineering Research Laboratory is that component of EPA's Research and Development program concerned with preventing, treating, and managing municipal and industrial wastewater discharges; establishing practices to control and remove contaminants from drinking water and to prevent its deterioration during storage and distribution; and assessing the nature and controllability of releases of toxic substances to the air, water, and land from manufacturing processes and subsequent product uses. This publication is one of the products of that research and provides a link between the researcher and the user community.

The particular study which is described here was aimed at improving the treatment of municipal wastewaters. Investigated were the potentials for improving anaerobic digestion of municipal wastewater sludge by adding specific enzymes in an effort to enhance the degradation of cellulose, oil, and grease.

Francis T. Mayo, Director
Water Engineering Research Laboratory

ABSTRACT

This study evaluates the effects of enzyme augmentation on municipal wastewater (MWW) sludge anaerobic digestion. The primary objective was to examine the impact of using enzymes to enhance the degradation of the cellulosic and the oil- and grease-rich sludge fractions.

The application of cellulase enzymes to enhance the anaerobic digestion of the cellulosic component of MWW sludge was evaluated in a series of laboratory-scale studies. Three modes of applying the enzymes were examined--as a pretreatment, post-treatment, and simultaneous treatment option with respect to anaerobic digestion. The pretreatment option incorporated a two-phase digestion process and was by far the most technically successful of the options examined. However, the advantages of this option were largely due to the two-phase process used to accommodate the pH requirements of the enzyme rather than to the enzyme additions. Compared with a single-phase digestion system, increases of 45 and 44 percent were observed for total solids reduction and methane production as a result of the two-phase digestion process alone. Addition of enzyme to the two-phase system further increased solids reduction and methane production to 60 and 66 percent.

Experimental data scaled to the size of the Xenia facility were compared with typical operations at a 15-day hydraulic-retention-time, single-phase digester system modeled on the Xenia facility. Results indicated that a net yearly savings of $11,133 with a 7-mo payback period for additional equipment could be achieved by using the two-phase digestion process without enzyme addition. To accomplish this result, the facility would need a minimal increase in tankage capacity. Based on the current cost of commercially available enzyme, an additional yearly savings of $1,525 would result from adding enzyme to the two-phase system. This savings could increase toward a maximum of $4,810 per year if enzyme costs could be reduced.

The application of lipase enzyme treatment to the oil and grease component of MWW sludge solids was evaluated in a similar series of laboratory experiments conducted with direct enzyme addition to a single-phase anaerobic digestion system operated at a 15-day hydraulic retention time. Compared with control conditions, increases of 2 and 4 percent were observed for total solids reduction and methane production as a result of lipase enzyme addition. Based on current enzyme costs, a net yearly savings of $701 would result from the use of lipase enzymes. No additional tankage capacity would be required for this process. Further increases could potentially be achieved by operating the system at a longer retention time.

The results of this program show that cellulase enzyme augmentation can produce a significant enhancement of the anaerobic digestion of municipal wastewater sludge. The largest effect noted, however, results from the use of a two-phase digestion process to accommodate enzyme addition. Under the conditions studied lipase enzyme has only a minimal effect on anaerobic digestion.

This report was submitted in fulfillment of Cooperative Agreement No. CR-810279-01-0 by the City of Xenia, Ohio, under sponsorship of the U.S. Environmental Protection Agency.

CONTENTS

Disclaimer ii
Foreword iii
Abstract iv
Figures ix
Tables xi

1. Introduction 1
   Background 1
   Program Objectives 1
2. Method of Approach 3
3. Literature Survey 7
   Literature Survey Results 7
      Cellulose Hydrolysis 7
      Grease/Oil Hydrolysis 10
   Enzyme Manufacturer Survey 11
4. Cellulase Enzyme Program 13
   Enzyme Study 14
   Pretreatment Process Concept 15
      Preliminary Study With Primary Sludge 15
      Optimization Study With Primary Sludge 23
      Process Study 29
   Posttreatment Process Concept 44
      Preliminary Study With Digested Sludge 44
      Optimization Study With Digested Sludge 48
      Process Study 53
   Simultaneous Treatment Process Concept 63
5. Lipase Enzyme Program 66
   Significance of Problem 66
   Enzyme Characterization With Artificial Substrate 66
   Enzyme Characteristics With Digester Skimmings 70
   Optimization Study With Digested Sludge 75
   Process Study 80
6. Technical and Economic Evaluation of Process Concepts 90
   Method of Comparison 90
   Xenia Glady Run Baseline Characteristics 90
   Cellulase Pretreatment Process Concept 92
      Technical Evaluation 92
      Economic Evaluation 97
   Lipase Simultaneous Treatment Process Concept 99
      Technical Evaluation 99
      Economic Evaluation 102

CONTENTS (continued)

7. Conclusions 104

References 105

Appendices

Appendix A. Experimental Methods 109

Appendix B. QA/QC Program and Date Summaries 112

FIGURES

Number		Page
1	Abbreviated work flow plan	3
2	Test and control digester system	5
3	Schematic of test and control digester systems	6
4	Proposed process concepts for use of cellulase enzymes	13
5	Activity assay results for Calbiochem-Behring and Novo enzyme products	16
6	Experimental design for Pretreatment Characterization Study	17
7	Reducing sugar production from cellulase enzyme treatment of primary sludge	20
8	Carbon dioxide concentration resulting from cellulase treatment of primary sludge	21
9	Hydrolysis of cellulose in raw primary sludge	22
10	Experimental design for Primary Sludge Optimization Study	24
11	Effect of incubation time on net cellulose hydrolysis at 35°C	26
12	Effect of incubation time on net cellulose hydrolysis at 40°C	27
13	Effect of incubation time on net cellulose hydrolysis at 50°C	28
14	Effect of incubation time on net cellulose hydrolysis	30
15	Net cellulose hydrolysis in Primary Sludge Optimization Study	31
16	Net cellulose hydrolysis in Primary Sludge Optimization Study at 35°C	32

FIGURES (continued)

Number		Page
17	Control and test systems for Cellulase Pretreatment Process Study	34
18	Pretreatment control system digester data	41
19	Pretreatment test system digester data	42
20	Reducing sugar production from cellulase enzyme treatment of digested sludge	47
21	Carbon dioxide content resulting from cellulase treatment of digested sludge	49
22	Hydrolysis of cellulose in digested sludge	50
23	Cellulose hydrolysis in digested sludge as a function of time	51
24	Control and test systems for Cellulase Posttreatment Process Study	54
25	Posttreatment control system digester data	60
26	Posttreatment test system digester data	61
27	Titration of fatty acids from soybean oil following 24-hr incubation	68
28	Saponification of soybean oil	69
29	Saponification of extracted skimmings oil	71
30	Composite temperature effect on fat hydrolysis of extracted skimmings oil	72
31	Effect of pH on hydrolysis of extracted skimmings oil	73
32	Enzyme concentration effects on oil/grease hydrolysis	74
33	Incubation time effects on oil/grease hydrolysis	76
34	Composite time/concentration effects on oil/grease hydrolysis	77
35	Hydrolysis of oil and grease in anaerobically digesting sludge	78

FIGURES (continued)

Number		Page
36	Methane production in anaerobically digesting sludge as a function of enzyme addition	79
37	Control and test systems for Lipase Simultaneous Treatment Process Study	80
38	Lipase test system digester data	86
39	Lipase control system digester data	87
40	Xenia Glady Run Facility	91
41	Simplified process flow diagram of Glady Run anaerobic digester system	95
42	Current and cellulase enzyme augmented Glady Run anaerobic digestion configurations	96
43	Current and lipase-augmented Glady Run anaerobic digestion configurations	100

TABLES

Number		Page
1	Computer Literature Search Results	8
2	Properties Of Candidate Enzymes For Cellulose Hydrolysis	11
3	Properties Of Lipase Enzyme	12
4	Assay Procedure Utilized To Compare Candidate Enzymes	15
5	Anova Results For Preliminary Study With Primary Sludge	18
6	Pretreatment Optimization Study Anova Results	25
7	Daily Volume Flows For Cellulase Pretreatment Process Study	35
8	Primary Sludge Characteristics For Pretreatment Process Study	35
9	Analysis Schedule For The Cellulase Pretreatment Process Study	36
10	Solids And Gas Production Data For The Pretreatment Process Study (Daily Basis)	37
11	Volatile Acids And pH In Pretreatment Process Reactors	38
12	Cellulose Analysis Of Primary Sludge And Reactor Effluents In The Cellulose Pretreatment Study	39
13	Mass And Energy Balances For Pretreatment Process Line	40
14	Analysis Of Test And Control Cellulase Pretreatment System Digesters For TKN And Total P	43
15	BOD, Filtration Studies, And Heating Value Analysis For The Pretreatment Process Studies	44
16	Performance Characteristics Of Pretreatment Process Systems	45
17	Anova Results For Preliminary Study With Digested Sludge	46

TABLES (continued)

Number		Page
18	Posttreatment Optimization Study Anova Results	52
19	Daily Volume Flows For Cellulase Posttreatment Process Study	55
20	Primary And Digested Sludge Feed Characteristics For Posttreatment Process Study	56
21	Analysis Schedule For The Cellulase Posttreatment Process Study	57
22	Solids And Gas Production Data For The Posttreatment Process Study (Daily Basis)	58
23	Mass And Energy Balances For Cellulase Posttreatment Process Line	59
24	Analysis Of Test And Control Cellulase Posttreatment Digesters For TKN And Total P	62
25	BOD, Filtration Studies, And Heating Value Analyses For The Posttreatment Process Study	63
26	Performance Characteristics Of Posttreatment Process Systems	64
27	Cellulose Analysis Of Digested Sludge Following 120-Hr Incubation At pH7 And 35°C	65
28	Experimental Conditions For Initial Lipase Study	67
29	Daily Volume Flows For Lipase Simultaneous Treatment Process Concept	81
30	Primary Sludge Characteristics For Lipase Simultaneous Line Study Process	82
31	Analysis Schedule For Lipase Simultaneous Treatment	83
32	Solids And Gas Production Data For The Lipase Process Study (Daily Basis)	84
33	Mass And Energy Balances For Lipase Simultaneous Treatment Line	85

TABLES (continued)

Number		Page
34	Analysis Of Test And Control Lipase Simultaneous Treatment Digesters For TKN And Total P	88
35	BOD, Filtration Studies, And Heating Value Analysis For The Lipase Simultaneous Treatment Process Studies	88
36	Performance Characteristics Of Lipase Process Systems	89
37	Data, Assumptions, And Reference Values Utilized To Calculate Mass And Volume Flows For The Glady Run Facility	93
38	Assumed Glady Run Operating Conditions	94
39	Xenia Baseline And Scaled-Up Cellulase Process Data Summary	97
40	Economic Assessment Of Cellulase Pretreatment Process Concept	99
41	Xenia Baseline And Scaled-Up Lipase Process Data Summary	101
42	Economic Assessment Of Lipase Simultaneous Treatment Process Concept	103

SECTION 1

INTRODUCTION

BACKGROUND

One of the most commonly employed treatment methods for municipal wastewater (MWW) solids is anaerobic digestion followed by one of a number of ultimate disposal options. This process is particularly advantageous in that it produces a sludge suitable for land application, efficiently removes pathogenic organisms, produces a potentially useful by-product fuel gas, and is generally accompanied by low operating costs (1). Despite these advantages, currently used anaerobic digestion technology has several operational and technical limitations. One of the most significant of these is the level of volume reduction achieved. Although anaerobic digestion reduces sludge volume and mass, the constantly growing cost and number of federal and state regulations governing the disposal of MWW sludges requires that current technology be optimized to produce the smallest amounts of sludge possible.

The objective of this program was to evaluate the application of enzyme augmention to current MWW anaerobic digestion practices. This concept was viewed as a method to enhance the sludge volume reduction and fuel gas production. Although commercial enzyme augmentation products are available for use in MWW treatment systems, an extensive search of the relevant experimental literature conducted in this program indicated very few useful data regarding their use. The present program was therefore designed to gather information on the use of enzymes specific for the degradation of the cellulosic and oil and grease fractions of MWW solids.

PROGRAM OBJECTIVES

The objectives of the first phase of this program were designed to investigate the use of cellulase enzymes specific for the hydrolysis of the cellulosic component of MWW primary sludge. Previous experience in the degradation of municipal solid waste has shown that cellulose is a relatively refractory compound to anaerobic biodegradation (2, 3). Since cellulose can comprise 15 to 35 percent of the total solids in primary sludge (4, 5),

enhancing its destruction could significantly improve current treatment practices. The objectives in this phase were therefore twofold:

Objective 1: To maximize the destruction of the cellulosic component of primary sludge and its subsequent conversion to methane-rich biogas.

Objective 2: To minimize the costs associated with an enzymatic hydrolysis/anaerobic digester system by optimizing process conditions.

In the course of the research program, it became increasingly clear that the oil and grease fraction of primary sludge is also a source of problems in the anaerobic digestion process. This problem manifests itself in the formation of a thick digester scum layer necessitating frequent digester maintenance. The problem is ubiquitous since grease and oil represent a significant portion of primary sludge total solids (4). To achieve the maximum benefit from this research program, the scope of work was modified to include an investigation of the potential of utilizing lipase enzyme technology to enhance the hydrolysis of oil and grease in primary sludge. The objectives of this Phase 2 effort were essentially identical to those listed previously for the research in cellulose hydrolysis.

The objective of the third and final phase of this program was to assess the impact of the research results on current operations at the City of Xenia, Ohio, Glady Run facility. This analysis was to include scaling up the laboratory data to permit an identification of the necessary modifications to implement an enzymatic hydrolysis/anaerobic digestion process at the Xenia facility. A technical and economic analysis of this process concept was also to be performed.

SECTION 2

METHOD OF APPROACH

To accomplish the stated objectives, a comprehensive research program was designed to investigate the use of enzymes in sludge treatment. Figure 1 shows an abbreviated work flow plan describing the major program objectives. The initial task in both the cellulose and oil and grease hydrolysis studies was the performance of a comprehensive computer-assisted survey of information available on this topic. Our next task was a careful evaluation of enzyme characteristics, emphasizing conditions relevant to optimum substrate hydrolysis. The final task included performance of studies to optimize the enzyme hydrolysis process and to evaluate the process concepts on an extended time basis.

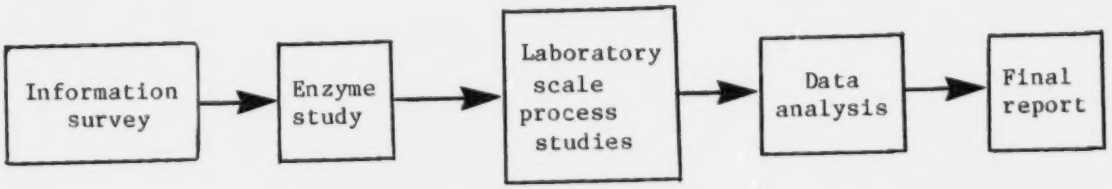


Figure 1. Abbreviated work flow plan.

The key to the performance of in this program consisted in following a carefully constructed set of experimental designs. Three major types of experiments were conducted to evaluate the enzymatic hydrolysis of cellulose, oil, and grease. The first class of experiments was directed at determining the impact of incubation time, temperature, pH, and enzyme concentration on substrate hydrolysis. The second class of experiments was designed to determine the optimum combination of these factors for maximizing substrate hydrolysis. These experiments utilized a factorial design in order to assess the main effects of the test variables as well as potential interactions between them (6,7). Results of these experiments were analyzed by analysis of variance techniques. The third class of experiments was designed to evaluate the enzyme treatment process concepts over an extended time period. All experiments were conducted using test and control laboratory-scale enzyme-reactor anaerobic digester systems. The digester systems, shown in

Figure 2 consisted of 6-L glass vessels maintained in a circulating water bath at 35°C. These vessels were connected to gas collection devices designed to function as spirometers, trapping the digester gas over an acidified, salt-saturated medium. Figure 3 shows a schematic drawing of the digester and gas collection systems. All data were referenced to standard conditions of 60°F and 30 in. Hg.

A technical and economic evaluation of applying enzyme technology at Xenia's Glady Run facility was accomplished using the data generated in the third class of experiments described previously. These data were extrapolated to the size of the Xenia facility in order to predict the effects of enzyme hydrolysis at a full-scale facility.

Throughout this program, standard analytical methodologies were utilized whenever possible. The majority of techniques were derived from either EPA's Methods for Chemical Analysis of Water and Wastes (8) or Standard Methods for the Examination of Water and Waste Water (9). Analytical methods not cited in these references are described in Appendix A. A comprehensive quality assurance/quality control (QA/QC) program was developed at the outset of this program. A discussion of this program as well as data summaries for the process studies are detailed in Appendix B to this report.

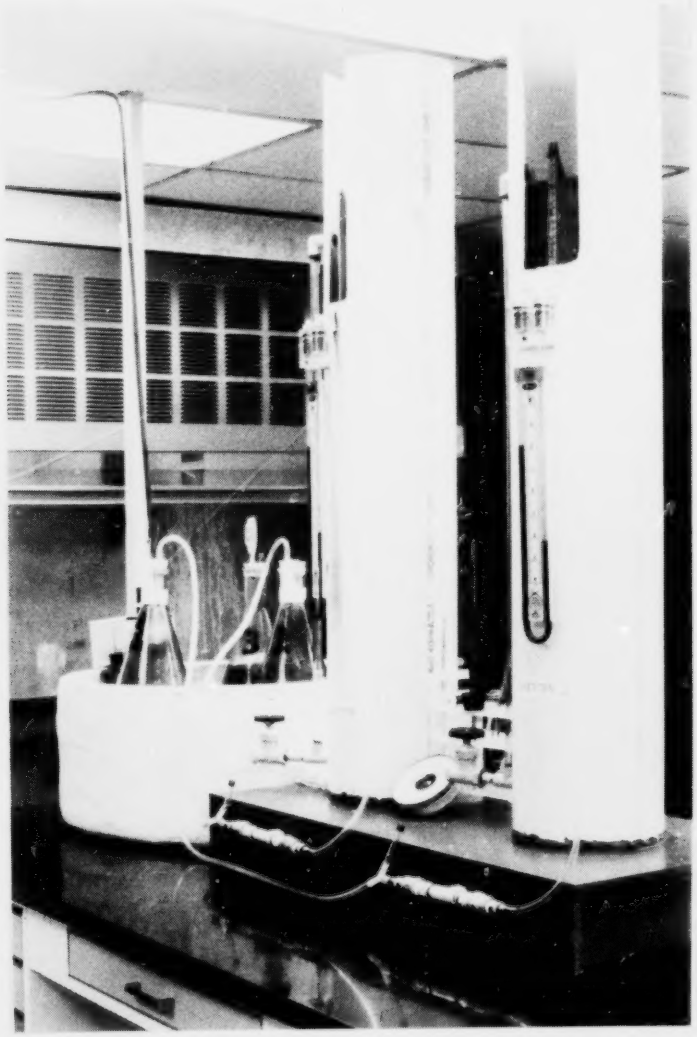


Figure 2. Test and control digester systems.

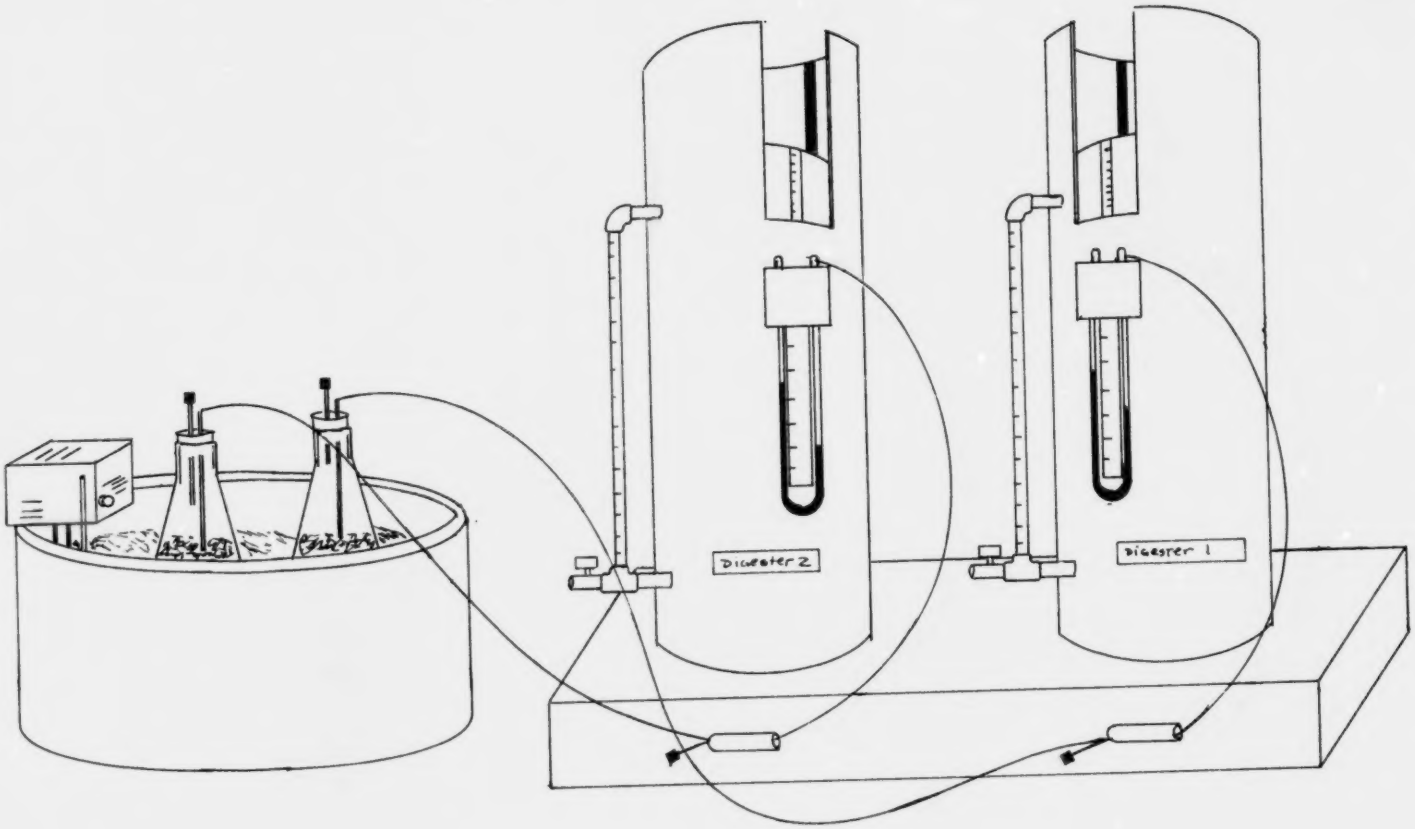


Figure 3. Schematic of test and control digester systems.

SECTION 3

LITERATURE SURVEY

LITERATURE SURVEY RESULTS

One of the first tasks in this program was a comprehensive survey of the available information on application of cellulase and lipase enzymes to anaerobic sludge digestion. This survey was conducted using the computer search facilities at the University of Dayton, Dayton, Ohio. Three separate data bases were searched using the intersection of sewage or anaerobic processes with either cellulase or lipase and protease as the search topics. The first data base searched was BRS/CHEM, which includes all listings from Chemical Abstracts from 1977 to date. The ENVIROLINE system, containing 106,000 records, was also searched from 1971 to date. The final data base searched was BRS/NTIS, which includes all listings contained in the National Technical Information Service from 1970 to date.

Table 1 summarizes the results of the computer data base search by category and number of citations identified. The following sections will discuss cellulase and lipase separately. In general, very little information directly applicable to enzyme augmentation of anaerobic digestion was found.

Cellulose Hydrolysis

No information directly applicable to the enhancement of cellulose hydrolysis in anaerobic processes by addition of exogenous enzyme sources was found in our literature survey. Nevertheless, a review of information relevant to cellulose hydrolysis is worthwhile in the presentation of program results. Cellulose is generally considered to be a material relatively resistant to biodegradation. Cellulose is composed primarily of linear chains of $\beta(1\rightarrow4)$ glucopyranose arranged into long bundles referred to as microfibrils. These bundles are assembled into higher order structures, often in association with other recalcitrant compounds such as lignins, pectins, and hemicelluloses. The molecular weight of cellulose ranges from about 50,000 to 2,500,000 in various species, with 300 to 15,000 glucose residues per molecule. As stated previously, cellulose can comprise 15 to 35 percent of the dry weight total solids in primary sludge (4,5).

The relatively high crystallinity and substitution with associated compounds of limited biodegradability make the hydrolysis of cellulose a difficult proposition. However, a number of biological processes are capable of cellulose hydrolysis. Thayer (10) examined the ability of various bacterial species to hydrolyze cellulose. A Pseudomonas sp. was identified

TABLE 1. COMPUTER LITERATURE SEARCH RESULTS

Data Base	Search topic	No. citations identified
BRS/CHEM	Cellulase/sewage or anaerobic	7
	Cellulose or cellulase/sewage or anaerobic	19
	Lipase/sewage or anaerobic	3
	Protease/sewage or anaerobic	2
	Total	31
Enviroline	Cellulose or cellulase/sewage or anaerobic	19
	Lipase or protease/sewage or anaerobic	7
	Total	26
BRS/NTIS	Cellulase/sewage or anaerobic	8
	Lipase or protease/sewage or anaerobic	2
	Total	10
	GRAND TOTAL	67

possessing both cell-bound and extracellular cellulase enzymes capable of hydrolyzing mesquite wood to 17 percent of its initial weight in 10 days at 30°C. This organism was also found to be relatively active against lignin and newsprint. Several Bacillus sp. and Brevibacterium sp. were also identified as capable of cellulose hydrolysis. The hydrolysis of cellulose by three thermophilic specifies was examined by Ben-Bassat (11): Thermoanaerobium brockii, Methanobacterium thermoautotrophicum, and Clostridium thermocellum. These organisms were shown capable of converting various hexoses, pentoses, and, in particular, cellobiose, an intermediate product of cellulose hydrolysis, to ethanol, lactate, acetate, and H_2, and CO_2. Only the C. thermocellum was capable of complete cellulose hydrolysis. In co-culture with C.thermohydrosulfuricum, C.thermocellum degraded 90 percent of a commercial cellulose substrate (48 to 96 hours), 50 percent of a delignified wood substrate (168 hours), and less than 1 percent of an aspen wood sample (168 hours). A number of studies of organisms isolated from sludge have also

shown cellulolytic potential. Khan et al (12), for example, have identified a mesophilic anaerobe of the Bacterioidaceae family capable of degrading cellulose to acetate, H_2, and CO_2, and reducing sugars.

One of the most studied sources of cellulolytic enzymes is the mesophilic fungi. Extensive research into the enzymatic digestion of cellulose by fungal enzymes has been conducted at the U.S. Army Natick Laboratories (13,14,15). One particularly well studied source is the fungus Trichoderma reesei. This fungal enzyme has been shown capable of converting cellulose to soluble sugars at very high efficiency (16). Enzymes derived from this source show three essential activities: an exoglucanase, removing reducing sugars from the end of the molecule; an endoglucanase, randomly hydrolyzing within the molecules; and a glucosidase, which hydrolyzes primarily cellobiose, a two glucose intermediate fragment of cellulose hydrolysis. The latter enzyme serves to prevent inhibition of cellulose hydrolysis by the end products cellobiose and glucose (17,18).

Synergistic relationships have been observed between the enzymes involved in cellulose hydrolysis. These relationships complicate the description of kinetic data. Mandels et al (19) have suggested that hydrolysis can be related directly to the log (enzyme/substrate). Novo laboratories has developed an empirical relationship between hydrolysis and (enzyme concentration × incubation time)$^{1/2}$ (20). One interesting study of the bacteria Ruminococcus albus has shown that in a defined medium there is a direct linear relationship between gas evolved and extent of cellulose hydrolysis (21).

Various methods of assaying the extent of cellulose hydrolysis as a function of enzyme action have been developed over the years. Canevascini et al (22) have reviewed the various assay procedures. They include carboxymethylcellulose to assay endoglucanase activity and insoluble celluloses to measure combined endo- and exoglucanase activities. The latter have included use of insoluble cotton, filter paper, or microcrystalline Avicel® cellulose. These assays show variable amounts of β-glucosidases are present in enzyme preparations, and at least one supplier suggests the need for supplementation with this enzyme (23).

As stated previously, very little information relating directly to cellulose hydrolysis and anaerobic digestion was found in the literature survey. The major work on cellulose hydrolysis and anaerobic digestion has been performed previously by SYSTECH using municipal solid waste/sludge systems (2,3,24). Some literature values indicate that typical anaerobic digestion is capable of 40 to 50 percent hydrolysis of cellulose (4). Stoichiometric calculations indicate that cellulose can be converted to methane relatively efficiently, yielding approximately 0.4 L methane per gram cellulose in 15 days (25).

Grease/Oil Hydrolysis

As with cellulose hydrolysis, very little survey information was found regarding the application of lipase enzymes to MWW treatment sludges. Only one particularly informative article was identified in which Wang et al (26) discussed the application of lipase enzymes as part of an ultrafiltration system for sewage treatment. The situation regarding oils and grease is complex because of the diversity of compounds in this class. The typical freon extractable oil and grease fraction contains biological as well as industrial contaminants such as certain organic dyes and related compounds (9). Within the biological lipid fraction can be found both simple and complex lipids (27). The simple lipids include terpenes, steroids, and other nonesterified compounds which are not susceptible to the class of lipase enzymes examined in this program. The complex lipids are primarily esterified forms of glycerol such as acylglycerols, phosphoglycerides, sphingolipids, and waxes. These compounds are among the saponifiable lipids, since they yield fatty acid salts (soaps) upon alkaline hydrolysis. Primary sludge has been reported to contain compounds of this class at 6 to 30 percent of the total solids (4).

The class of enzymes examined in this program included the acylglycerol hydrolases. This group of enzymes has been well studied because of their importance in mammalian metabolism. The fungi are also a major source of lipase enzymes. Various Aspergillus sp. and Mucor sp. have been characterized for the presence of acylglycerol hydrolases (28,29). The enzyme derived from Mucor miehei--chosen for use in this program--has a specificity for the one and three positions of triglycerides and has been shown to degrade 70 percent of beef tallow to fatty acids and glycerol at 2 percent loading and 40°C in a 24-hr period (30). The majority of assay procedures currently applied to lipase hydrolysis measure the extent of hydrolysis as a function of the saponifiable fatty acids released (29,31). Typical substrates included in these assays are olive oil or purified acylglycerols such as tributyrin.

As stated previously, little information relating lipid hydrolysis to anaerobic digestion was found. Some literature values exist indicating that typical anaerobic digestion is capable of 50 to 60 percent hydrolysis of fats (4). However, discussions with various plant operators suggest that grease and oil can be a serious problem for anaerobic digesters, resulting in scum layer formation and accompanying transfer problems. McCarty has shown that fatty acids are among the slower compounds to degrade in anaerobic digesters (25). On the other hand, the chemical structure of some fats is such that high methane yields can be expected. Using standard stoichiometric equations it can be shown, for example, that compounds such as tripalmitoyl glycerol or pig lard can be expected to yield on the order of 1 L methane per gram of material in 15 days (25).

ENZYME MANUFACTURER SURVEY

As stated previously in Section 3 of this report, the major objective of the manufacturer survey was to identify candidate cellulase enzymes for this program. Table 2 summarizes the relevant parameters of enzymes obtained from Calbiochem-Behring, Sigma, and Novo Laboratories Inc. The most obvious difference between the products resides in the assays used to measure enzyme activity levels. It is extremely difficult to relate the results of one assay to another when they are based on separate measures of cellulose hydrolysis.

TABLE 2. PROPERTIES OF CANDIDATE ENZYMES FOR CELLULOSE HYDROLYSIS

Supplier	Enzyme	pH optimum	Temp. optimum	Activity*	Assay method†
Calbiochem-Behring	Cellulysin§	4.5	40°C	10,000 units/g	1
Sigma	Cellulase§	5.0	37°C	0.1 to 0.2 units/mg	2
Novo Laboratories Inc.	Celluclast 200 L§	4.8	50°C	200 C_1u/g	3
Novo Laboratories Inc.	Novozym 188**	4.8	50°C	250 Cbu/g	4

* Activity is a measure of an enzyme's ability to convert substrate to product under specific assay conditions in terms of units/wt enzyme.

† Assay method:

1. One unit is a measure of decomposing activity on filter paper at 40°C, pH 4.5.
2. One unit will liberate 1.0 µmole of glucose from Avicel® cellulose in 1 hr at pH 5.0 at 37°C (2-hr incubation time).
3. One unit will liberate 1 µmole/min reducing carbohydrate from Avicel® at pH 4.8 and 50°C (20-min incubation time).
4. One unit will liberate 1 µmole/min glucose from cellobiase at pH 4.8 and 50°C.

§ β-1,4-glucan cellobiohydrolases acting on cellulose (endo- and exoglucanases).

** β-1,4-glucanohydrolase acting on cellobiose (glucosidase).

It was therefore decided to examine each of these products using a common assay and base the decision of which enzyme to use on this information as well as on cost and availability data.

Major interest in lipase enzymes did not develp until late in this program. To obtain a maximum amount of data in the remaining time it was decided to conduct all experimentation using a Novo Laboratories product, Novozyme 225. Table 3 summarizes pertinent aspects of this enzyme product. The enzyme is produced by fermentation of a selected strain of Mucor miehei. It has a 1,3--specificity with optimum activity for substrates containing short chain fatty acids, but retains two-thirds of this activity with longer chain fatty acids (29).

TABLE 3. PROPERTIES OF LIPASE ENZYME

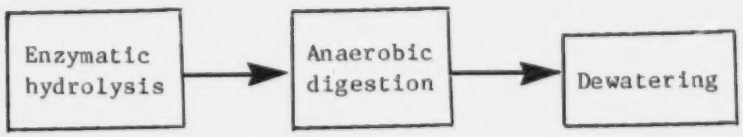
Supplier:	Novo Laboratories Inc.
Product:	Novozyme 225, Mucor miehei product
pH optimum:	~7 to 8
Temperature optimum:	~40°C
Activity:	13,300 Lu*/g
Assay Method:	Titration of liberated fatty acids with incubation at 30°C and pH 7.0
* Lu:	Amount of enzyme which liberates 1 µmole butryic acid per minute from a tributyrin substrate in a pH-stat at 30°C, pH 7.0

SECTION 4

CELLULASE ENZYME PROGRAM

The basic work flow plan for the cellulase enzyme program was shown previously in Figure 1. Within this framework three potential process concepts for the use of cellulase enzymes in primary sludge treatment were evaluated. Figure 4 illustrates the concepts examined. In the first option the use of cellulase was viewed as a pretreatment option to maximize hydrolysis prior to anaerobic digestion. The second option examined cellulase usage as a posttreatment process following the digester. The third option involved enzyme treatment simultaneous with digestion.

Option 1:



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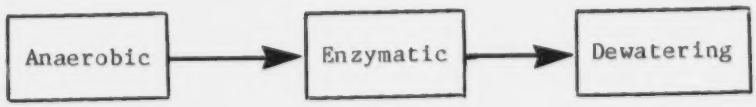


Option 3:

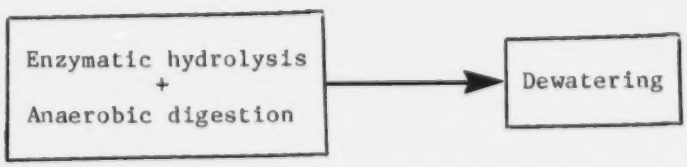


Figure 4. Proposed process concepts for use of cellulase enzymes.

ENZYME STUDY

The first task of the cellulase enzyme program was the identification of an enzyme product suitable for use in this program. Three candidate cellulase enzymes, each supplemented with a separate glucosidase, were evaluated for their ability to enhance the hydrolysis of cellulose in primary sludge. As stated previously in Section 4, manufacturers' information on the relative activity of the products using different assay procedures was extremely difficult to interpret. It was therefore necessary to develop and conduct a standard evaluation procedure to determine the enzyme of choice for this program. The major criteria for acceptance were the ability of the enzyme to degrade cellulase under the conditions examined in this program and the anticipated cost and availability of the enzyme as a commercial product.

Previous literature studies of the application of cellulose enzymes identified several methods of evaluating enzyme activity utilizing substrates such as filter paper, cotton, carboxymethylcellulose, and Avicel® (22,32). Each of these methods measures a specific aspect of cellulase activity which may or may not be applicable to the degradation of natural cellulosic materials. It was decided to employ an assay using Avicel® microcrystalline cellulose as substrate. Assays of this type have been shown to relate well to the ability of the enzyme to degrade native cellulosic materials such as those present in sludge (22). They are also relatively easy to accomplish using standard laboratory equipment.

The assay procedure utilized to compare the three candidate enzymes is detailed in Table 4. The candidate enzymes were evaluated at enzyme/substrate ratios ranging up to 10^5 ppm by weight or 10 percent by weight. In all cases the cellulase enzymes were augmented with a cellobiase enzyme, Novozym 188, to maximize the production of glucose as an end product. The cellobiase was added to the celluclast enzymes at a 1:10 weight ratio as suggested by manufacturer's information (23).

Figure 5 illustrates the reducing sugar produced from cellulose hydrolysis as a function of enzyme concentration and incubation time. Data are presented for the NOVO and Calbiochem-Behring enzyme products. Results from our analysis of the Sigma enzyme are not included here since under the conditions of our assay, this enzyme did not produce an equivalent extent of cellulose hydrolysis. The data are plotted with reducing sugar as a function of $(\text{enzyme concentration} \times \text{incubation time})^{1/2}$. The basis for this empirical relationship has been developed by NOVO Laboratories Incorporated as a method to describe the kinetics of cellulose hydrolysis over wide ranges of substrate concentration and incubation time (33). Figure 5 clearly shows that both enzyme products yield a linear increase in cellulose hydrolysis as a function of time and concentration. Linear regression analysis of the data indicated fitted equations of:

Calbiochem: $\text{product} = 0.107\ (\text{conc.} \times \text{time})^{1/2} - 6.608$
Novo: $\text{product} = 0.042\ (\text{conc.} \times \text{time})^{1/2} - 2.477$

TABLE 4. ASSAY PROCEDURE UTILIZED TO COMPARE CANDIDATE ENZYMES

Assay substrate:	Avicel® microcrystalline cellulose
pH control:	pH 4.8 with 0.1-molar acetate buffer
Incubation:	20 min in a Lab-Line® Orbit Environ-Shaker at 3.4 Hz with 2-cm displacement
Temperature:	50°C
Enzyme substrate concentrations:	$0-10^5$ ppm (wt) 0-10 percent (wt)
Product measurement:	Colorimetric determination of reducing sugars (see Appendix A)

for the Calbiochem and NOVO enzyme products, respectively. On the basis of these data alone, the Calbiochem enzyme produced the highest level of cellulose hydrolysis at a given concentration and time.

Criteria for evaluating enzyme performance also included consideration of the economic impact of enzyme usage. Since the CalbiochemBehring enzyme product is a relatively highly purified, research grade enzyme, its cost and availability were considered prohibitive for the present use. The NOVO Laboratories enzyme is currently produced on a commercial scale at purity levels suitable for food grade uses. Even though it produced a lower extent of hydrolysis, its significantly lower cost and availability in bulk quantities led to selection of this product as the enzyme of choice for further study in this program.

PRETREATMENT PROCESS CONCEPT

In the examination of cellulase enzyme usage as a pretreatment concept, three major experimental tasks were accomplished. These included a preliminary study to characterize the enzyme performance with primary sludge and an optimization study to examine the effects of time, temperature, and concentrations. The final task was an in-depth process study using cellulase enzymes.

Preliminary Study With Primary Sludge

The basic format of the preliminary characterization study is shown in Figure 6 detailing the experimental design. The major test effects monitored

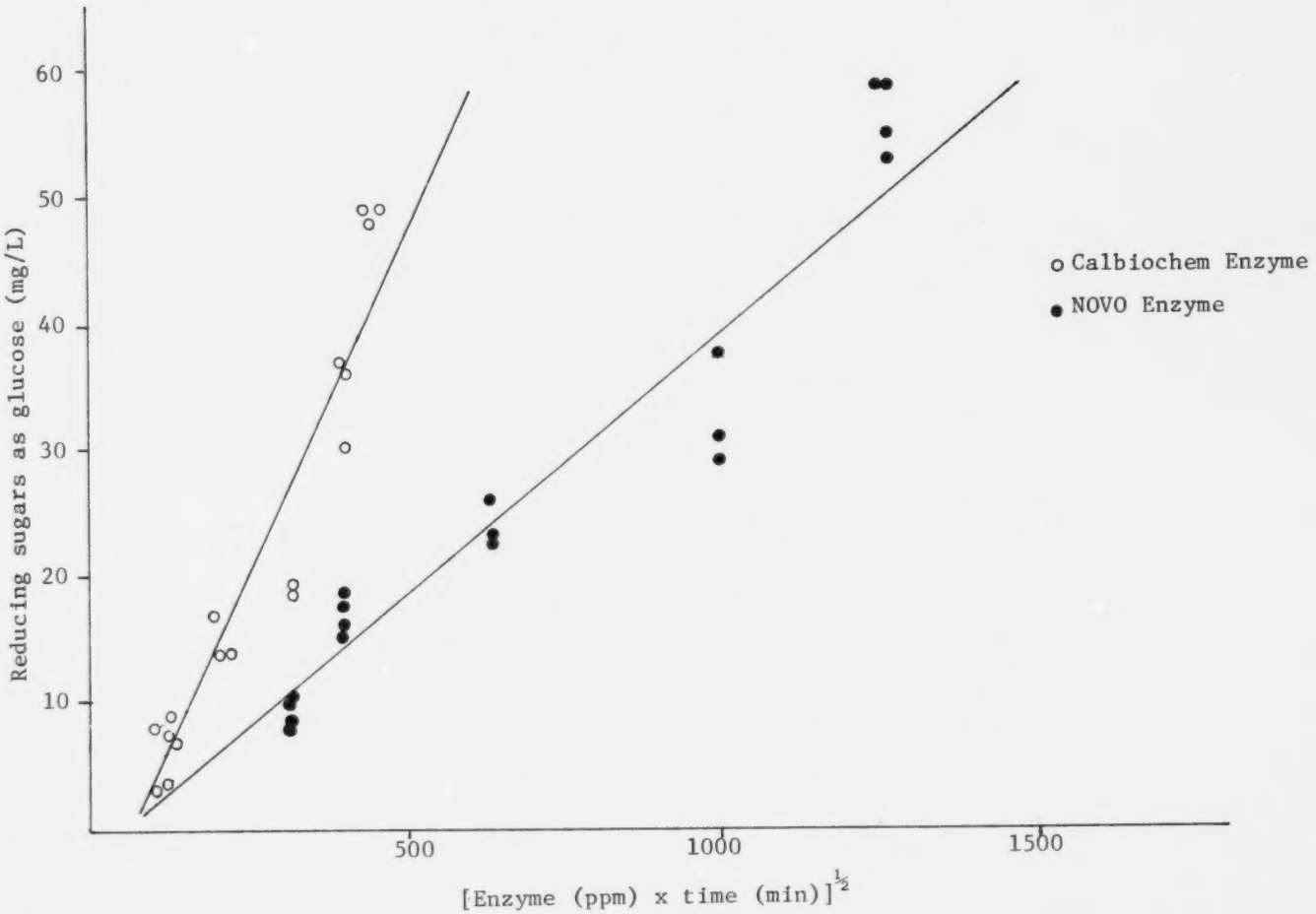


Figure 5. Activity assay results for Calbiochem-Behring and NOVO enzyme products.

Retention time (hours)	Enzyme concentration (ppm by wt) 0	10^2	10^4	10^6
24	X	X	X	X
48	X	X	X	X
72	X	X	X	X

X: Test effects measured were reducing sugar production, gas production, and cellulose hydrolysis.

Figure 6. Experimental design for Pretreatment Characterization Study.

in response to enzyme addition were reducing sugar production, gas production, and cellulose hydrolysis. Methods for these analyses are discussed in the appendix. These effects were monitored in order to determine an appropriate index of cellulase activity with primary sludge for use in this program. The overall experimental objective was to determine the effects of incubation time and enzyme concentration on cellulose hydrolysis. A series of 250 mL flasks were loaded with 100 mL of primary sludge from the Xenia Glady Run plant. The sludge (2.66 percent total solids, 81.96 percent volatile solids) was previously adjusted with HCl to a pH of 4.8, the reported enzyme optimum (23). Cellulase and cellobiase were then added in a 10:1 weight ratio to give final cellulase/sludge total solids concentrations of 0, 10^2, 10^4 and 10^6 ppm by weight. The flasks were purged with nitrogen to replace dissolved gases and incubated with a water gas trap at 35°C in a Lab-Line® Orbit Environ-Shaker at 3.4 Hz with 2-cm displacement. A temperature of 35°C was chosen to conform with normal anaerobic digestion temperatures. Subsets of the flasks consisting of triplicate for the control and each enzyme loading level were removed after a 24-, 48-, and 72-hr incubation.

Table 5 shows the results of the analysis of variance (ANOVA) of incubation time and enzyme concentration effects on reducing sugar production, gas production, and cellulose hydrolysis. The purpose of

TABLE 5. ANOVA RESULTS FOR PRELIMINARY STUDY WITH PRIMARY SLUDGE

Analysis	Source of variance	Sum of squares	Degrees of freedom	Mean square	Computed f	f Critical (α = .05)
Reducing sugar (mg/L)	Time	242,697.0	2	121,348.5	7.00	3.89
	Conc	2,326,490.2	3	775,496.7	44.74	3.49
	Time × conc	141,945.3	6	23,657.6	1.37	3.00
	Error	208,010.0	12	17,334.2		
		2,919,142.5	23			
Gas (percent CO_2)	Time	274.4	2	137.2	33.7	3.89
	Conc	4,522.4	3	1,507.5	370.4	3.49
	Time × conc	235.7	6	39.3	9.7	3.00
	Error	4.9	12	4.0		
		5,037.4	23			
Cellulose hydrolysis (percent of initial hydrolyzed)	Time	589.87	2	249.9	24.99	4.26
	Conc	9,250.22	2	4,625.1	391.83	4.26
	Time × conc	1,390.48	4	347.6	29.45	3.63
	Error	106.24	9	11.8		
		11,336.81	17			

applying ANOVA was to partition the total variation of the experiment into meaningful components that could be related to the particular levels and combinations of incubation time and enzyme concentration studied. The following sections discuss the impact of these variables on the test effects measured.

The results of the reducing sugar analysis of the flasks contents showed no significant interaction between time and concentration at $\alpha = .05$; i.e., the computed f value was not greater than the critical f value. Both time and enzyme concentration did, however, show significant effects on reducing sugar production. Figure 7 represents the results of reducing sugar analyses. With the exception of the highest enzyme loading, all flasks showed an increase in reducing sugar after 24-hr incubation which either remained relatively constant or declined with further incubation. In the case of the highest enzyme loading, a steady decline in reducing sugar was observed. These results are similar to previous findings regarding the hydrolysis of cellulose in a non-sterile environment. With high enzyme loadings, reducing sugars are released in large quantities and rapidly met by an increase in the bacterial population. It should also be noted that there is a minimal amount of sugar in the Novo enzyme product which leads to a significant initial time zero concentration at high enzyme loading. This leads to an equally rapid uptake of reducing sugars from the medium. At lower enzyme concentration, reducing sugars are released at a slower rate and the rate of bacterial growth and uptake of reducing sugars is therefore reduced. Reducing sugars are then capable of increasing until the growth rate can match the increased sugar concentration. Although levels of reducing sugar were found to be directly responsive to enzyme concentration and incubation time, the non-linear response to incubation time limits the usefulness of this parameter in gauging the extent of cellulose hydrolysis.

Table 5 also summarizes the ANOVA results of gas analyses performed in this experiment. The gas data reported represents the percent replacement of nitrogen in the initial purge gas by carbon dioxide, which should be proportional to the metabolism of the microorganisms present. The data indicate that significant effects ($\alpha = .05$) were observed in response to both incubation time and enzyme concentration as well as their combination. Figure 8 illustrates the gas data as a function of incubation time at the various enzyme loadings. These data tend to parallel the responses shown in Figure 7 for reducing sugars. That is, gas replacement with carbon dioxide rises for the first 24 hours and then stabilizes as the reducing sugar levels also decline.

Table 5 also shows the ANOVA for cellulose hydrolysis as a function of incubation time and enzyme concentration. The data showed significant effects ($\alpha = .05$) in response to both incubation time and concentration as well as their combination. By far the most easily interpreted response in cellulose hydrolysis occurs as a function of enzyme loading as shown in Figure 9. The data are plotted in this figure as the log of cellulase concentration (wt enzyme/wt sludge total solids basis) versus net cellulose hydrolysis. Net cellulose hydrolysis refers to the destruction occurring over

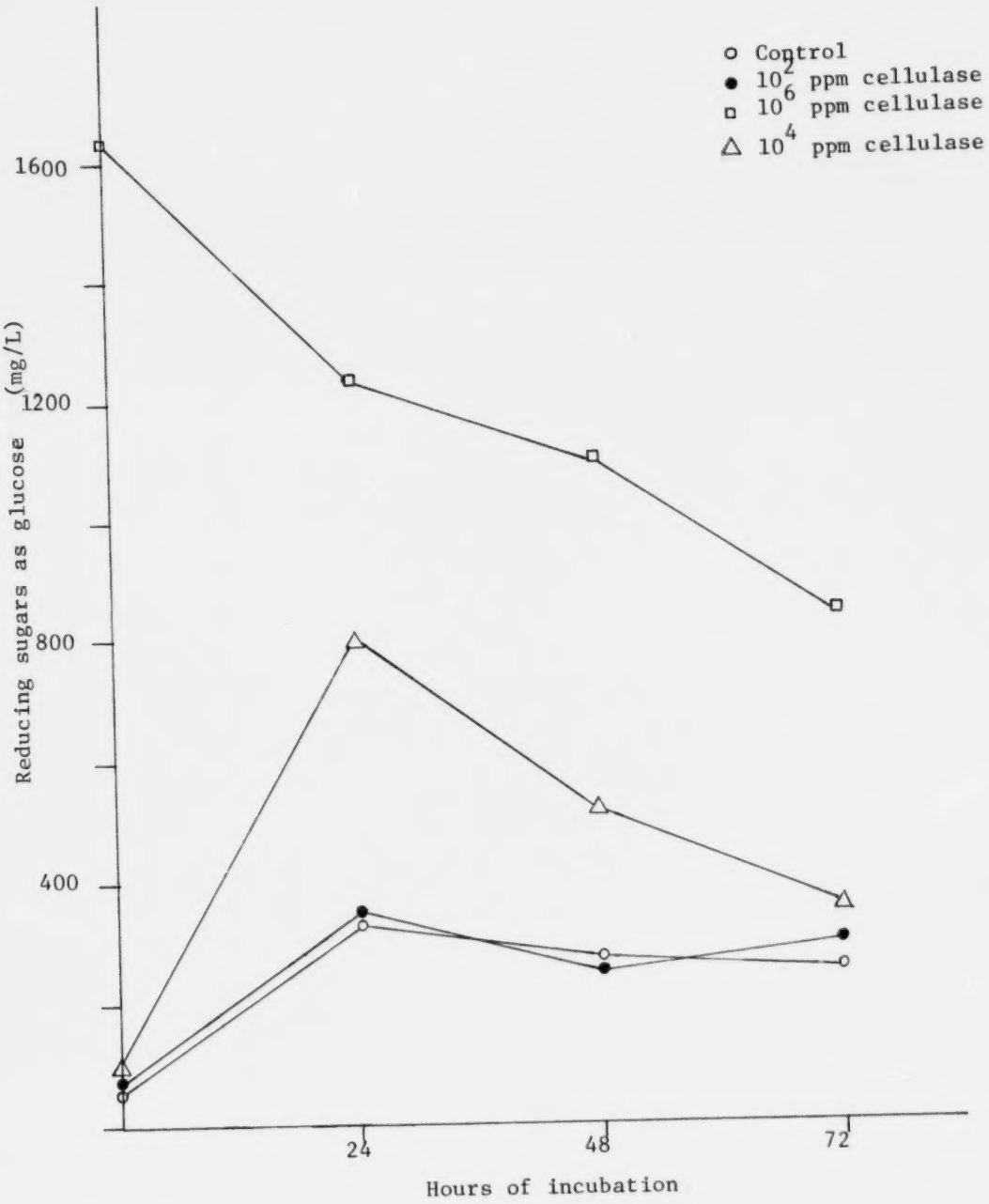


Figure 7. Reducing sugar production from cellulase enzyme treatment of primary sludge.

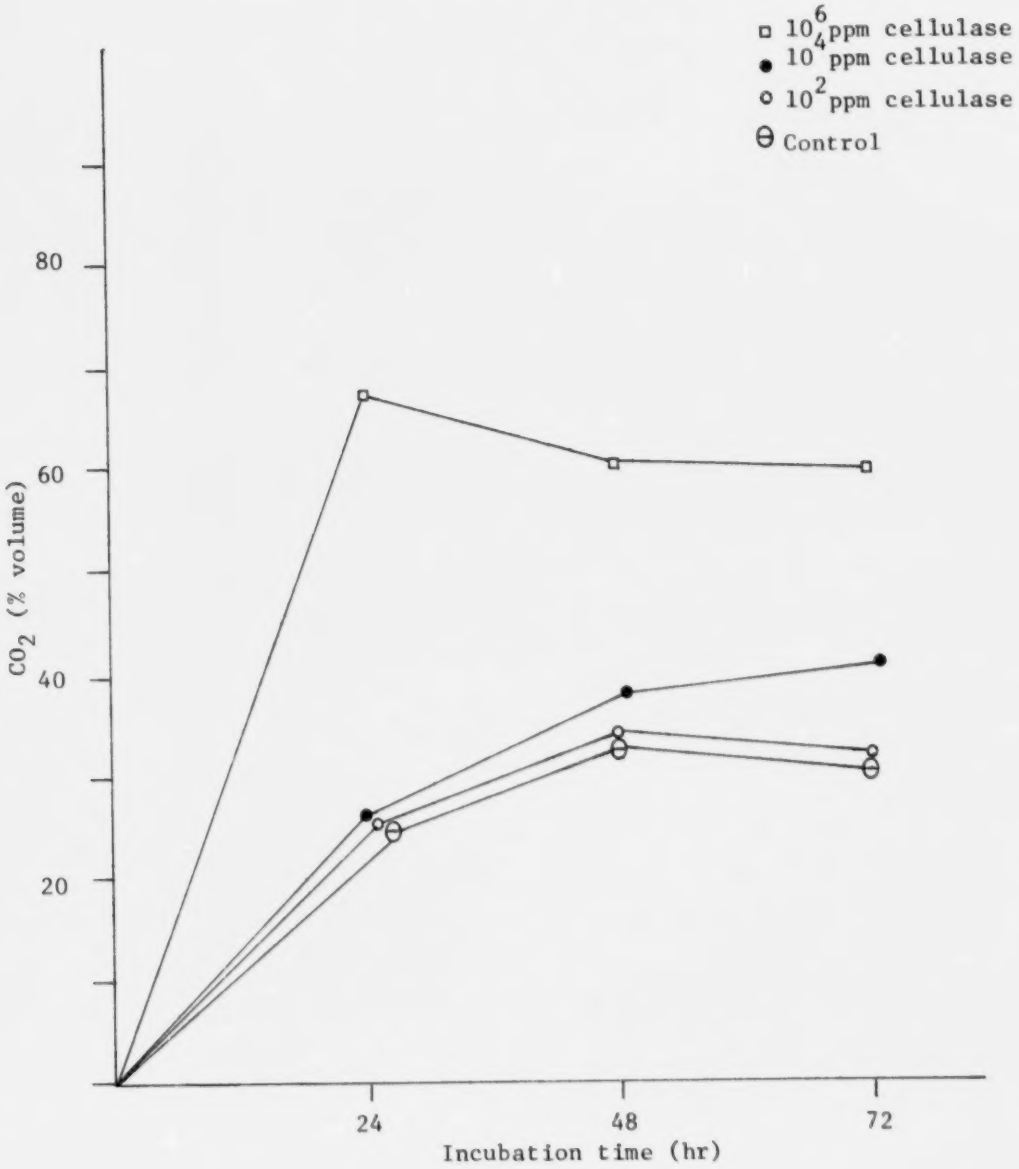


Figure 8. Carbon dioxide concentration resulting from cellulase treatment of primary sludge.

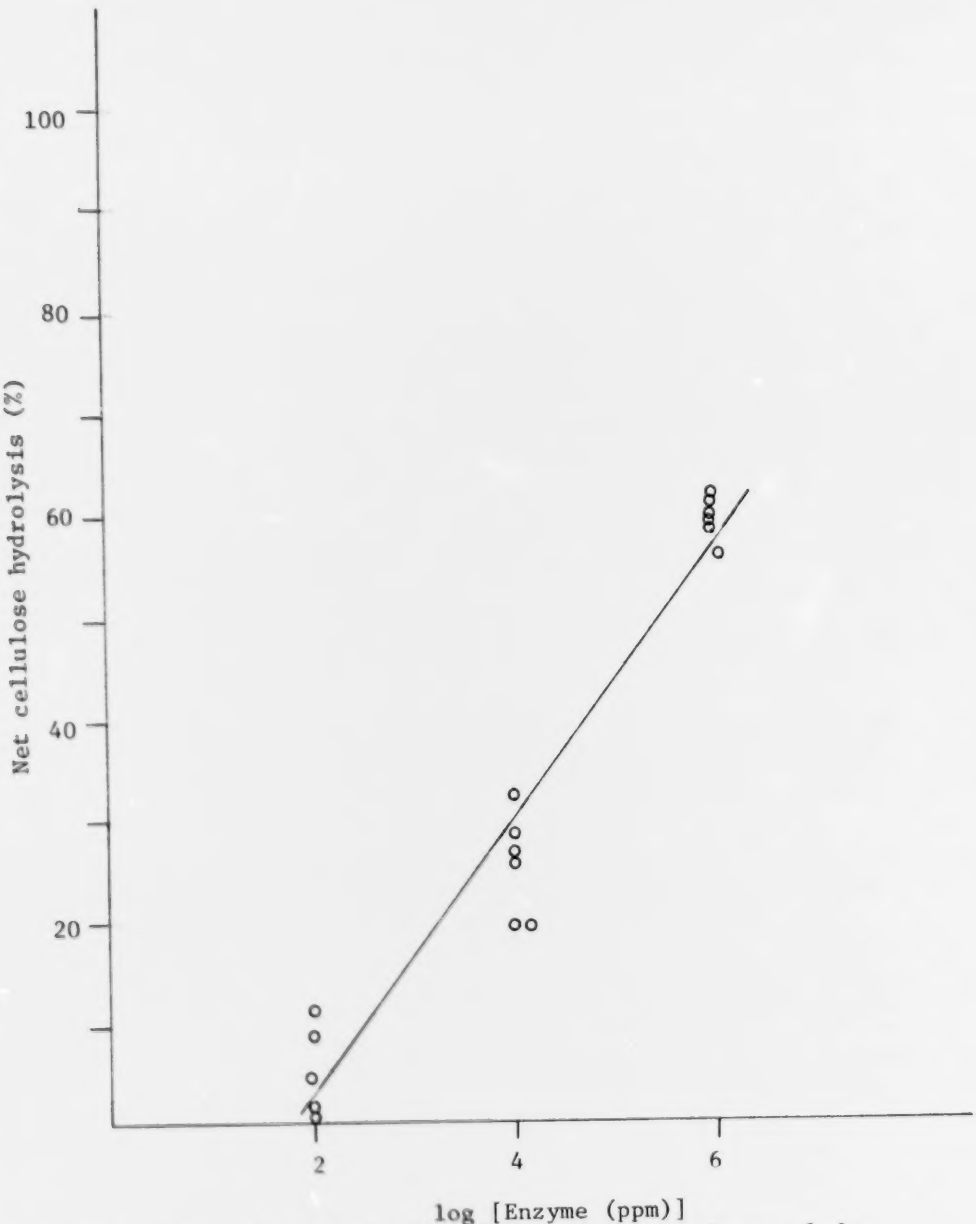


Figure 9. Hydrolysis of cellulose in raw primary sludge.

and above that observed in blank controls containing no added enzyme but incubated for the same time interval (24, 48, or 72 hour). Figure 9 shows that a linear relationship can be drawn between net cellulose destruction and log (enzyme concentration). The equation fitted to this line by linear regression is

net cellulose destruction = 13.2 log (cellulase ppm) - 22.2,
with a correlation coefficient (r) of 0.97.

The results of the preliminary study with primary sludge showed that incubation time and enzyme concentration had significant effects on all of the test effects measured. However, the most easily interpreted response to these variables was found to occur with the extent of cellulose hydrolysis. This study therefore identified cellulose hydrolysis as the test effect to be monitored in the subsequent optimization study.

Optimization Study With Primary Sludge

The objective of the optimization study with cellulase enzyme application to primary sludge was to determine an optimum combination of incubation time, temperature, and enzyme concentration for maximum cellulose hydrolysis. Figure 10 illustrates the factorial experimental design applied for this study.

Two deviations were made from the previous study in order to maintain tighter control over the inherent variability of the primary sludge. These included prescreening the primary sludge through a 9.5-mm sieve to remove the larger particles (cigarette filters, gum, foil, etc.) which interfered with reproducibly dispensing small portions of sludge. The sludge (including that used in the controls) was also buffered at pH 4.8 with 0.1 M potassium hydrogen phthalate (KHP) to prevent excursions in pH which interfere with enzyme hydrolysis. This measure was warranted for a tightly controlled laboratory study, but would not be practical for a full-scale application of cellulase enzyme technology.

The experiment was conducted by loading a series of 250-mL flasks with 100 mL of primary sludge from the Xenia Glady Run plant. The sludge (3.41 percent total solids, 22.4 percent cellulose) was buffered at pH 4.8 using 0.1 M KHP. Cellulase and cellobiase were added in a 10:1 weight ratio to give final cellulase/sludge total solids concentrations of 0, 10^2, 10^3, and 10^4 ppm by weight. The flasks were then purged with nitrogen to remove dissolved gases and incubated with a water gas trap in a Lab-Line® Orbit Environ-Shaker at 3.4 Hz with a 2-cm displacement. Subsets of the flasks (preheated to the incubation temperature before enzyme addition) were incubated at 35, 40, and 50°C for 1, 8, and 16 hours. Each subset consisted of a triplicate for the control and each loading level. Incubation time intervals were set at 1, 8, and 16 hours for two reasons. First, the preliminary primary sludge experiment reported above showed that cellulose hydrolysis did not dramatically increase over the range from 24- to 72-hr

		Enzyme Concentration (ppm) (wt enzyme/wt sludge total solids)											
		0			10^2			10^3			10^4		
Time (hr)		1	8	16	1	8	16	1	8	16	1	8	16
Temperature (°C)	35	X	X	X	X	X	X	X	X	X	X	X	X
	40	X	X	X	X	X	X	X	X	X	X	X	X
	50	X	X	X	X	X	X	X	X	X	X	X	X

X: Test effect measured was cellulose hydrolysis.

Figure 10. Experimental design for Primary Sludge Optimization Study.

incubation. Second, since incubation time would obviously have a significant impact on the economics of this process, a minimum incubation period was desirable.

Table 6 presents the results of the ANOVA performed on the experimental data using cellulose hydrolysis as the master variable responsive to time, temperature, and concentration. Using an $\alpha = .05$ as a critical value, the following single factors showed a significant effect on cellulose hydrolysis: incubation time and enzyme concentration. None of the potential interactions showed a significant effect ($\alpha \leq .05$) on cellulose hydrolysis. The following sections describe the effects of incubation time, temperature, and enzyme concentration.

The effect of incubation time on net cellulose hydrolysis at various enzyme concentrations is shown in Figures 11, 12, and 13. These figures illustrate the data obtained at 35°, 40°, and 50°C, respectively. At 35° and 40°C there is an obvious linear increase in hydrolysis as a function of incubation time. An increase in slope of the fitted linear regression lines is also observed as a function of increasing enzyme concentration. These relationships are most obvious at 35°C and become progressively weaker at 40° and 50°C. It seems likely that this phenomenon is related primarily to the health and stability of the microorganisms present in the primary sludge.

TABLE 6. PRETREATMENT OPTIMIZATION STUDY ANOVA RESULTS

Analysis	Source of variance	Sum of squares	Degrees of freedom	Mean squares	Computed f	α
Weight cellulose hydrolyzed	Temp	.4275E-02	2	.2138E-02	3.29	.7274E-01
	Conc	.5048E-01	3	.1683E-01	25.87	.1592E-04
	Temp × conc	.4513E-02	6	.7521E-03	1.15	.3894
	Time	.1207E-01	2	.6033E-02	9.28	.3671E-02
	Temp × time	.7564E-02	4	.1891E-02	2.91	.6781E-01
	Conc × time	.8174E-02	6	.1362E-02	2.09	.1299
	Time × temp × conc (error)	.7805E-02	12	.6504E-03		

Levels of test variables:

Temperature	50°, 40°, 35°C
Enzyme concentration (wt enzyme/wt sludge total solids)	0, 10^2, 10^3, 10^4 ppm
Time	1, 8, 16 hr

Deviation from the optimum temperature for microbial growth (near 35°C) results in a decreased level of microbial metabolism, which indicates that the microorganisms are less able to remove the products of cellulose hydrolysis. Previous work has shown that accumulation of glucose, an end product of cellulose hydrolysis, is capable of feedback inhibition of cellulose hydrolysis (3,34). A limited analysis of glucose levels in the flasks at 40° and 50°C showed that glucose concentrations ranging from 0.5 to 1.2 percent were observed. Such levels have been observed to produce up to a 50 percent decrease in cellulose hydrolysis (3). The important conclusion regarding these data is that incubation at 35°C appears to be more appropriate than higher temperatures for cellulose destruction in primary sludge.

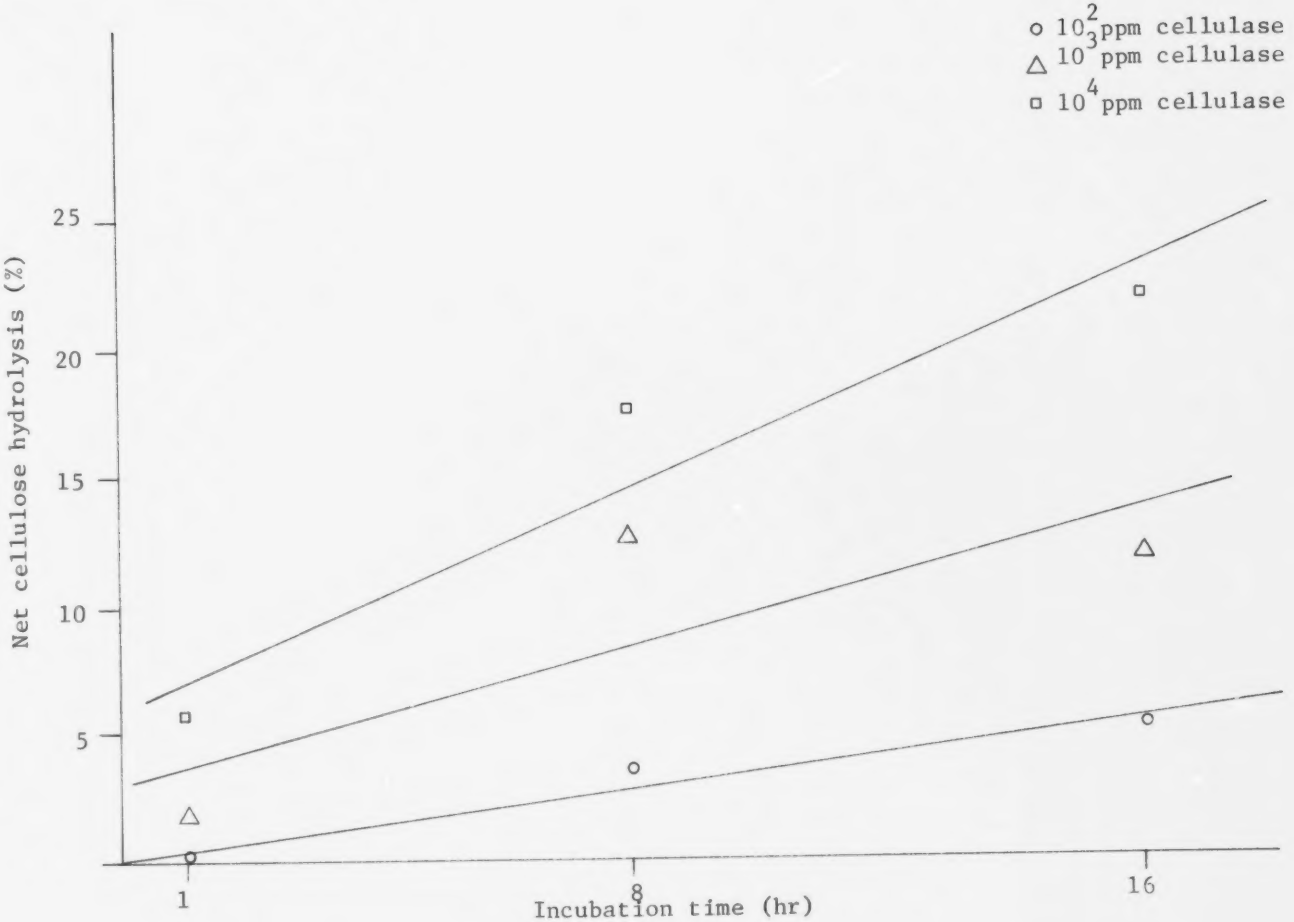


Figure 11. Effect of incubation time on net cellulose hydrolysis at 35°C.

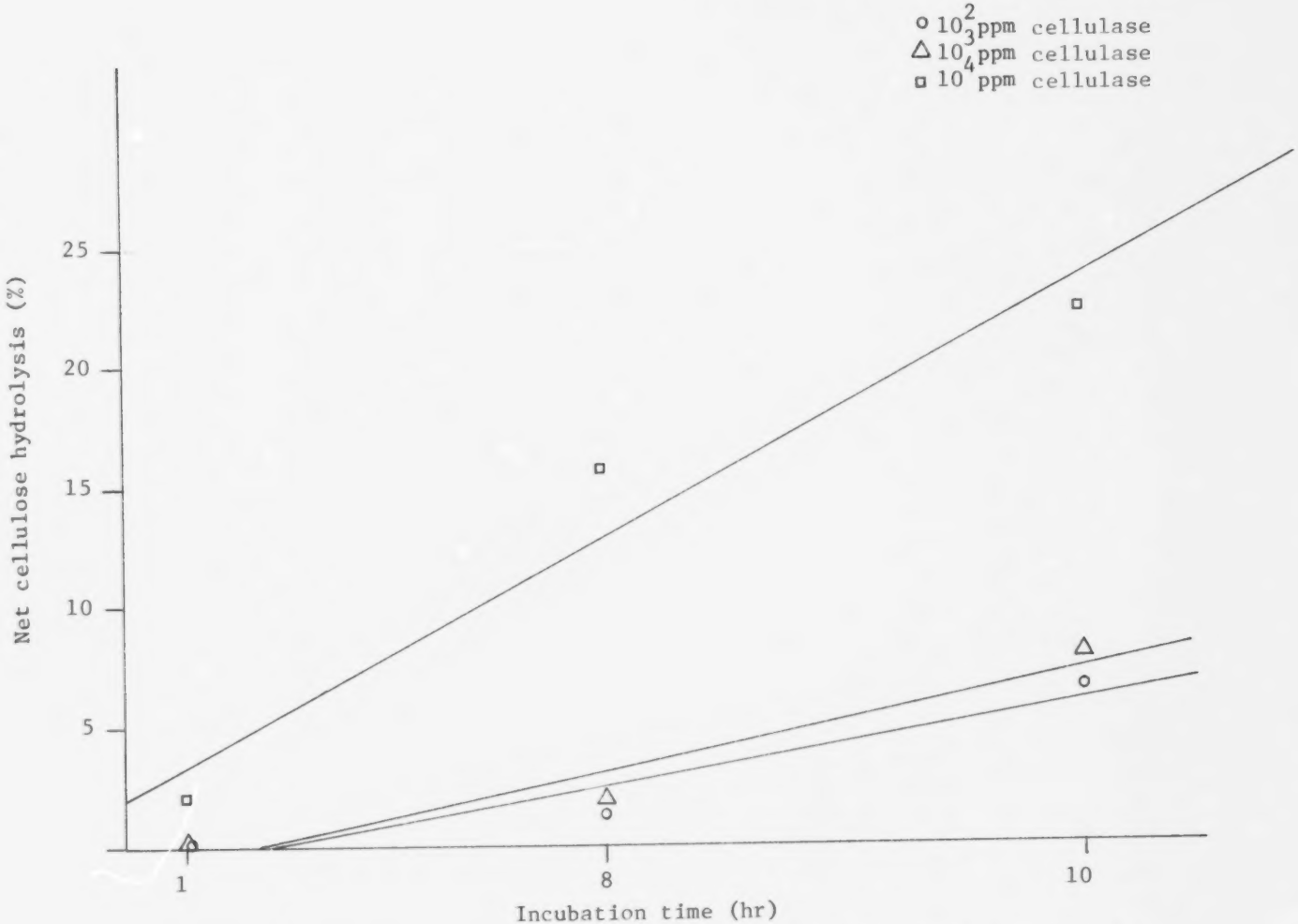


Figure 12. Effect of incubation time on net cellulose hydrolysis at 40°C.

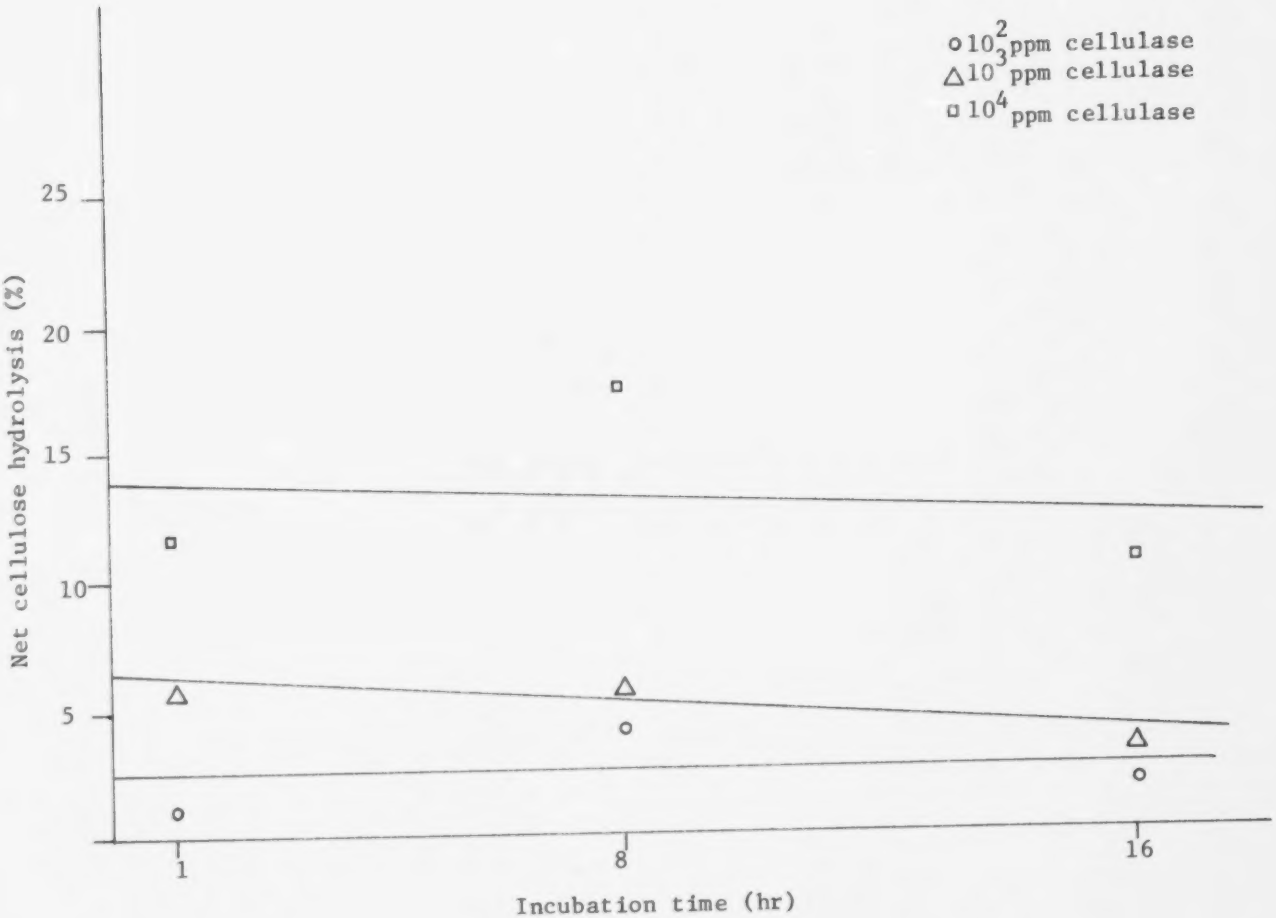


Figure 13. Effect of incubation time on net cellulose hydrolysis at 50°C.

Figure 11 shows that incubation time has a strong positive effect on cellulose hydrolysis at 35°C. In order to further clarify the question of an optimum retention time, data at the same enzyme loading level from the preliminary and optimization studies on cellulose hydrolysis in primary sludge at 35°C were assembled. Figure 14 shows the relationship between incubation time and cellulose hydrolysis at a loading of 104 ppm cellulase/primary sludge total solids. At an incubation time of 16 hours, a net hydrolysis of approximately 20 percent is achieved, which is nearly 70 percent of the hydrolysis observed at 72 hours. Since retention time is a highly significant factor in commercial application of enzyme technology, 16 hours was chosen as the retention time to be used in subsequent experiments.

The results of the ANOVA for the optimization study shown previously in Table 5 indicated a high level of confidence ($\alpha < .0002$) in the effect of enzyme concentration on net cellulose hydrolysis. Figure 15 shows a compilation of the test data reporting net cellulose hydrolysis as a function of (enzyme concentration × time)1/2. This empirical relationship has been described previously in this report as a means to predict net cellulose hydrolysis over wide ranges of incubation time and enzyme concentration. An excellent relationship can be drawn using this equation, yielding a linear regression correlation coefficient (r) of 0.91 when data from the 35°, 40°, and 50°C runs are examined together. However, since 35°C was previously identified as more appropriate than higher temperatures for cellulose destruction in primary sludge, it is useful to look at the data gathered at 35°C separately. Figure 16 shows this relationship. A linear regression analysis of this data yields a predictive equation for net cellulose hydrolysis as a function of (incubation time × concentration)1/2

$$\text{Net cellulose hydrolysis} = .049\ (\text{hour} \times \text{ppm cellulase})^{1/2} + 3.12$$
$$r = 0.93$$

The results of the optimization study with primary sludge yielded three major conclusions regarding the use of cellulase as a pretreatment process. First, a temperature of 35°C appears more appropriate than higher temperatures. Second, an incubation of 16 hours will achieve significant hydrolysis and yet not be prohibitively long for a commercial process. Third, enzyme concentration and incubation time can be used to predict the extend of cellulose hydrolysis with a high level of confidence. In order to ensure that the subsequent process study could demonstrate a measurable effect, a nominal value for (concentration × time)1/2 was arbitrarily chosen at 200. This was expected to produce a predicted net cellulose destruction of approximately 13 percent. It was also decided not to alter the pH of the primary sludge, since its normal value of approximately 5 is relatively close to the enzyme optimum of pH 4.8.

Process Study

In order to evaluate the cellulase pretreatment process concept over an extended time interval a laboratory-scale process study was conducted.

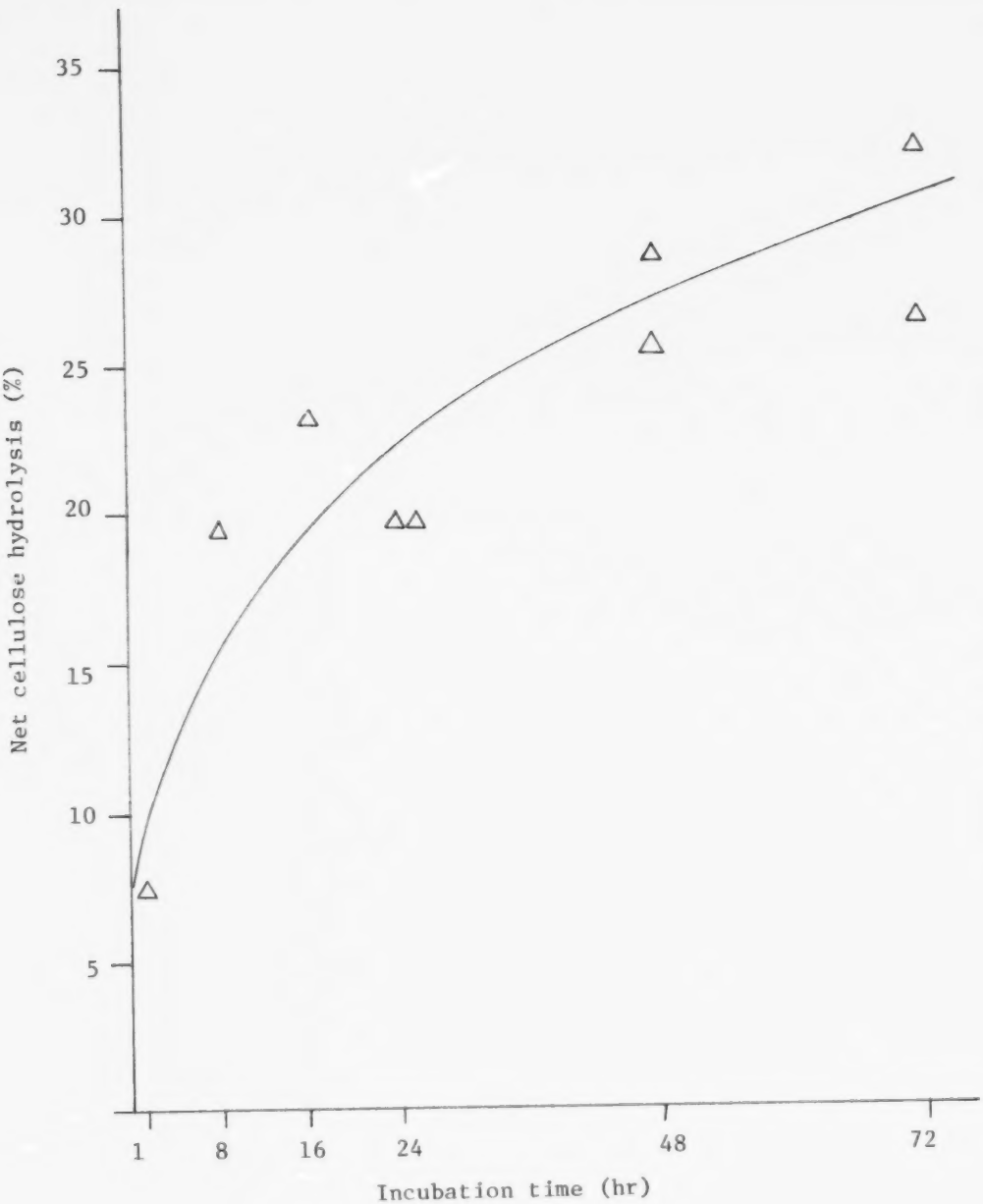


Figure 14. Effect of incubation time on net cellulose hydrolysis.

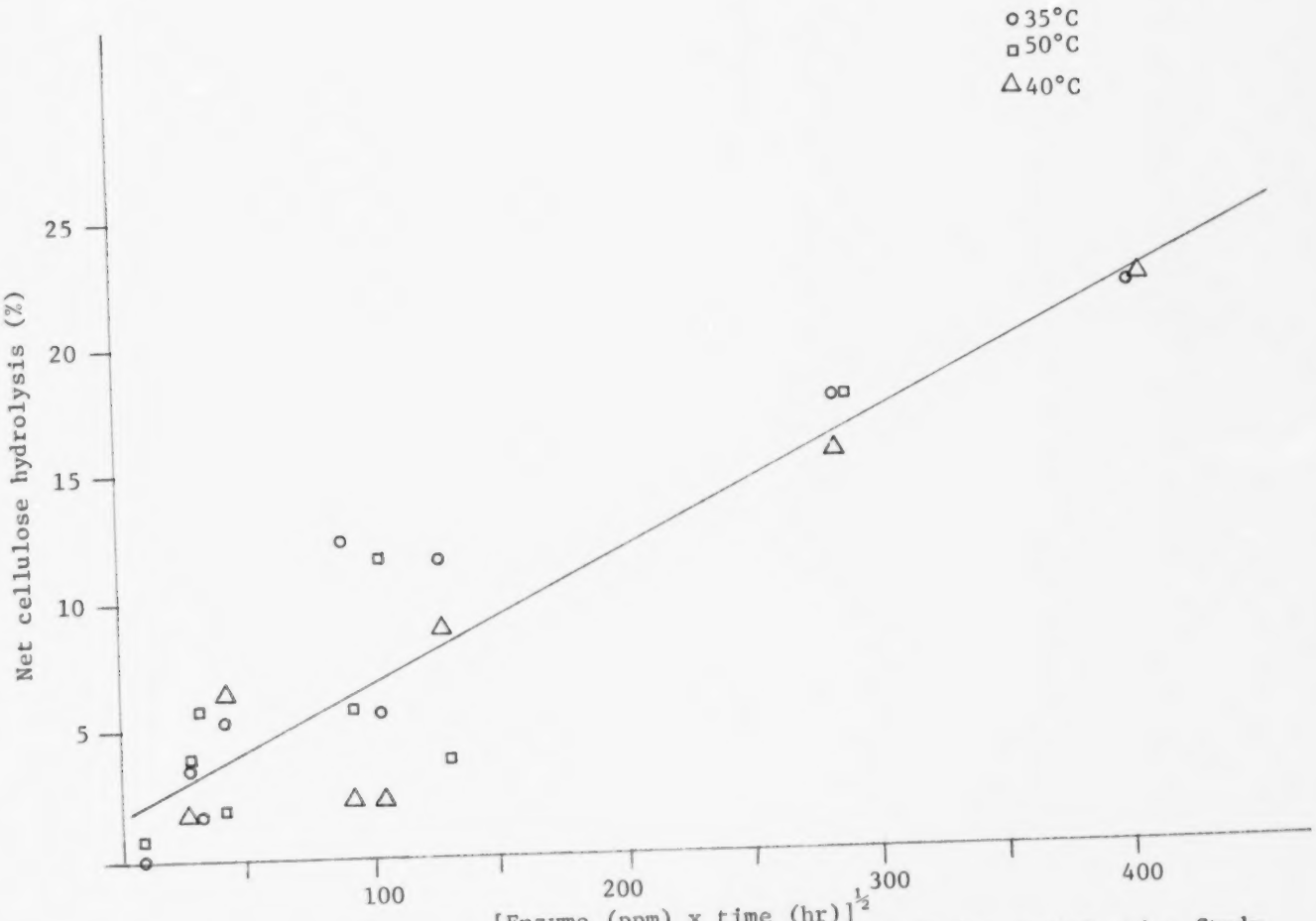


Figure 15. Net cellulose hydrolysis in Primary Sludge Optimization Study.

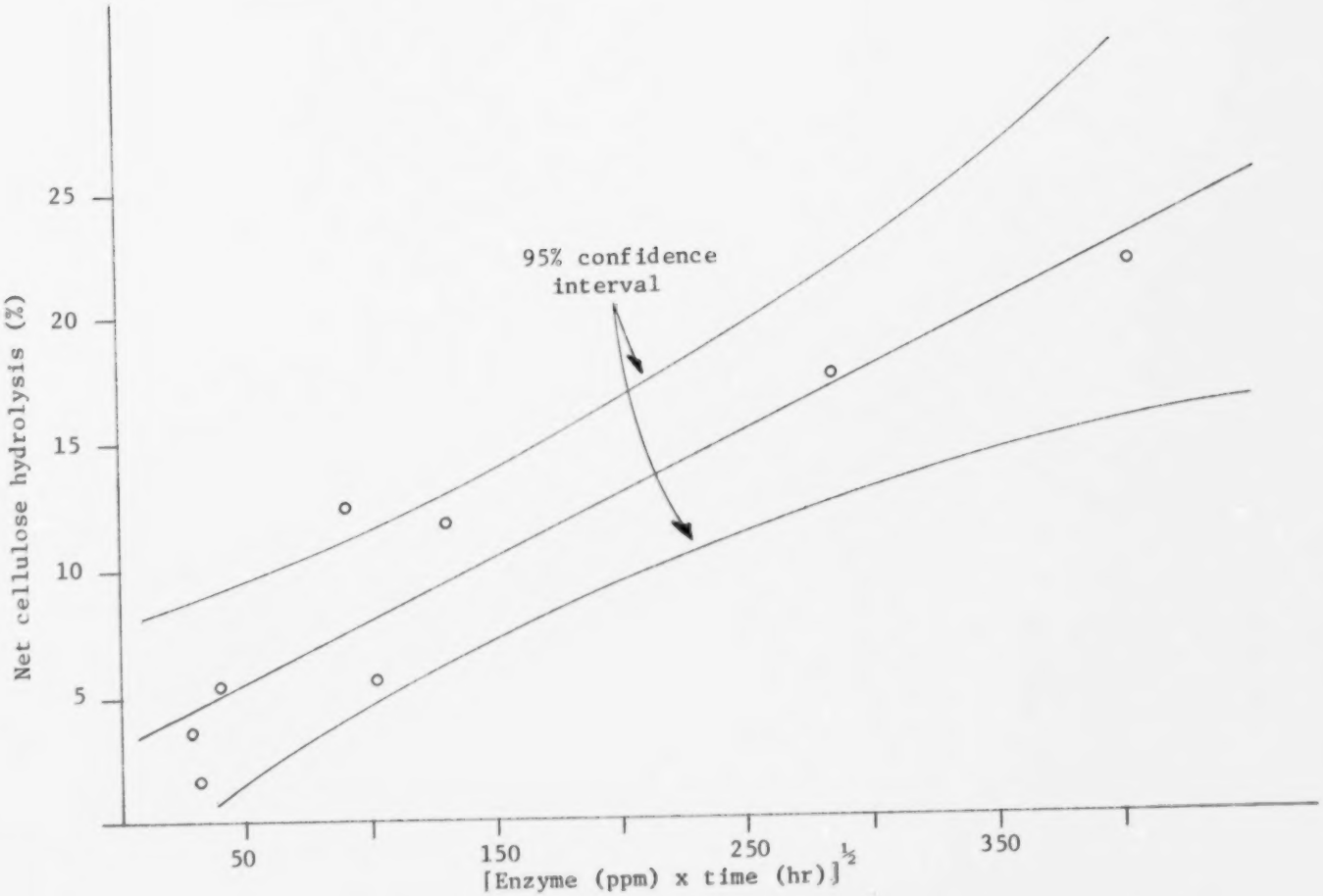


Figure 16. Net cellulose hydrolysis in Primary Sludge Optimization Study at 35°C.

Figure 17 provides a schematic illustration of the two laboratory systems employed for this evaluation. The control system differed from the test system in that inactive, heat-denatured enzyme was added to the control, while the test system received active enzyme. The pretreatment reactors consisted of 500-mL flasks incubated in a batch mode for 16 hours at 35°C in a Lab-Line® Orbit Environ-Shaker at 3.4 Hz with 2-cm displacement. The digesters, shown in Figure 2 were 6-L glass flasks equipped with gastight stoppers connected to gas collection devices by Tygon tubing. The digesters were maintained in a circulating water bath at 35°C and operated at a 15-day hydraulic retention time. The gas collection spirometers were constructed of PVC pipe and filled with an acidified, salt-saturated gas collection solution. The spirometers were calibrated to permit standard gas volume measurements (60°F and 30 in. Hg).

The test systems were operated as semi-continous flow processes. Table 7 summarizes the daily volume flows through the systems. A consistent method of operating the test and control systems was established in order to provide as much reproducibility as possible in this experiment. To control the input solids to the reactors, the primary sludge was filtered through a 9.5-mm sieve and adjusted daily to approximately 2.5 percent total solids by dilution with tap water. This value is within the normal range cited for primary sludge (2 to 7 percent) and yet dilute enough to permit easy handling (4). Characteristics of the feed sludge are shown in Table 8. Sludge samples were obtained fresh three times weekly and held at 4°C prior to use. The actual reactor volumes were maintained slightly in excess of 300 mL to provide aliquots for the required analyses. The digesters were sampled and fed and gas production recorded once each day at approximately 1200 hours. The digesters were thoroughly mixed by shaking three times each day at approximately 0800, 1200, and 1700 hours.

The experimental design employed to conduct the process study was formulated to test the following hypothesis: is the performance of the test system better than that of the control? Several measures were employed to permit a valid assessment of this hypothesis. First, before any testing was begun the test and control system was operated identically with no enzyme addition for at least two hydraulic retention times (30 days). It was required that solids destruction and gas production in the systems not be significantly different ($\alpha = .05$) during this period. Second, this same procedure was conducted following the testing interval to ensure that no differences unrelated to enzyme addition had developed. Finally, all data taken during the testing phase were analyzed on a daily or weekly basis utilizing a t-test for paired observation with an $\alpha = .05$ (7). This approach was taken to minimize the effects of the day-to-day variability in primary sludge composition on subsequent systems performance. During this study, enzyme was added to the systems at a mean concentration of 2930 ppm by weight (0.29 percent by weight).

Table 9 summarizes the analyses performed to evaluate the test hypothesis for the pretreatment process study. The various analyses listed were performed to permit evaluation of solids destruction and gas production,

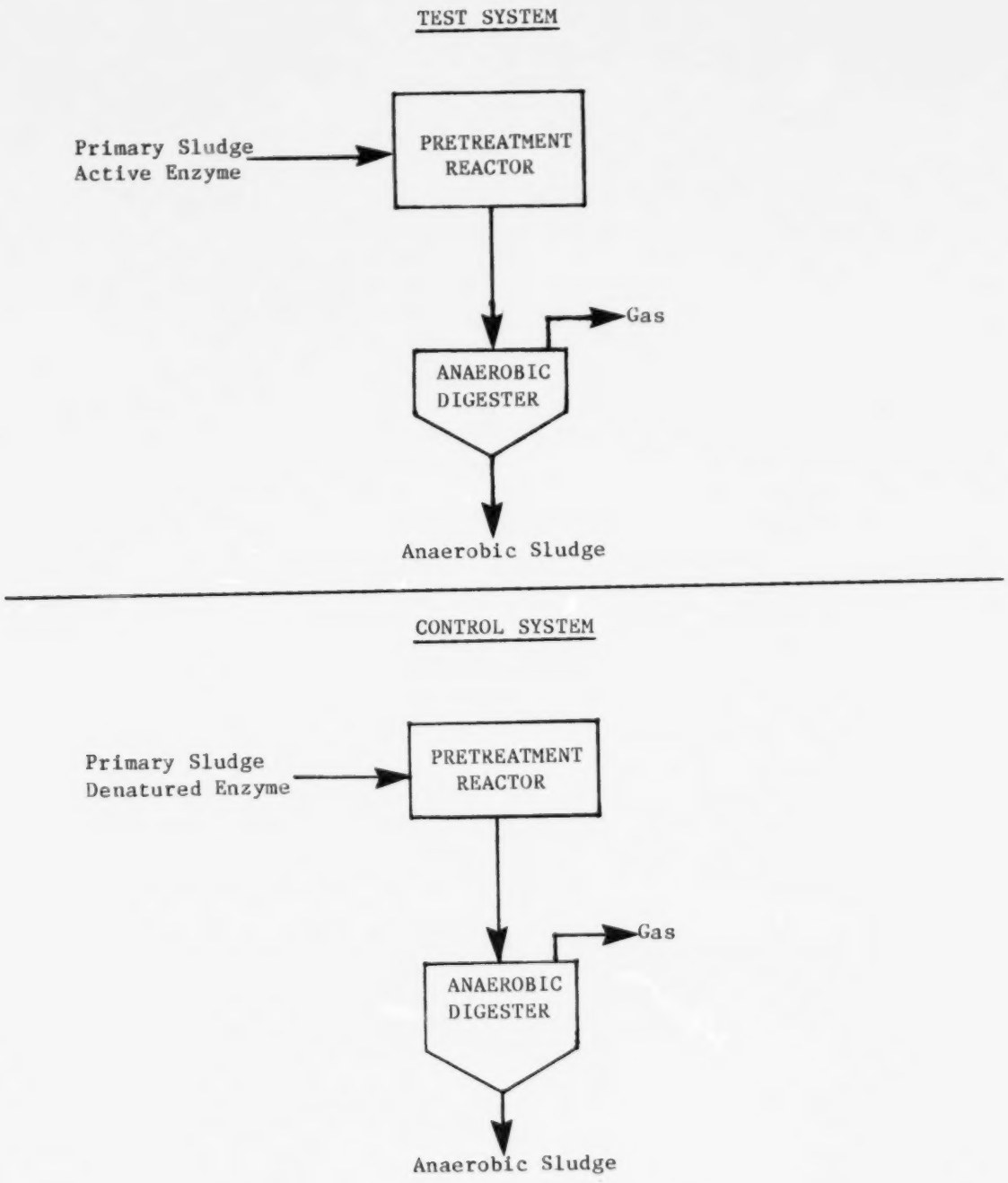


Figure 17. Control and test systems for Cellulase Pretreatment Process Study.

assessment of general system health, and calculation of mass and energy balances for scale-up to a commercial scale process. The latter analysis is described in more detail later in this report.

TABLE 7. DAILY VOLUME FLOWS FOR CELLULASE PRETREATMENT PROCESS STUDY

Stream	Control system (mL)	Test system (mL)
Primary sludge	300	300
Enzyme	10*	10
Reactor effluent	300	300
Digester influent†	300	300
Digester effluent	300	300

* Heat-denatured enzyme.
† Digesters were maintained at 4500 mL with a 15-day hydraulic retention time.

TABLE 8. PRIMARY SLUDGE CHARACTERISTICS FOR PRETREATMENT PROCESS STUDY

Characteristic	Mean Value
Total solids (TS) (percent)*	2.52
Total volatile solids (TVS) (percent of TS)	83.5
Cellulose (percent of TS)	19.6
pH	5.4
Heat content (Btu/lb)	7490

* Adjusted by dilution with tap water.

TABLE 9. ANALYSIS SCHEDULE FOR THE CELLULASE PRETREATMENT PROCESS STUDY

		TEST SYSTEM						CONTROL SYSTEM					
Analysis	Xenia primary sludge	Reactor in	Reactor out	Digester in	Digester out	Filter cake	Filtrate	Reactor in	Reactor out	Digester in	Digester out	Filter cake	Filtrate
TS	D		MWF		MWF	C			MWF		MWF	C	
VS	D		MWF		MWF	C			MWF		MWF	C	
pH	D		D		D				D		D		
Gas volume					D						D		
CH_4 percent					D						D		
CO_2 percent					D						D		
Volume	D	D	D	D	D		C	D	D	D	D		C
Weight	D	D	D	D	D	C		D	D	D	D	C	
Enzyme weight		D						D					
Volatile acids			C		C				C		C		
Alkalinity					C						C		
TKN					C						C		
Total P					C						C		
BOD					CX						CX		
Btu/lb	C				C	C					C	C	
Filter yield					C						C		
Specific resistence					C						C		
Cellulose	C		C						C				

D = daily
MWF = Monday, Wednesday, Friday
C = weekly composite
CX = weekly composite, analysis performed by Xenia
VS = Volatile solids
TKN = total Kjeldahl nitrogen

Table 10 summarizes the mean values for the solids and gas production data obtained during the pretreatment process study. All test data are shown in the appendix. The duration of the study was 21 days. Also included in this table is a statement regarding the presence of statistically significant (α = .05) differences between the control and test systems data. This information was determined by computing daily differences between the systems and applying a paired t-test (7).

TABLE 10. SOLIDS AND GAS PRODUCTION DATA FOR THE PRETREATMENT PROCESS STUDY (Daily Basis)

Process stream	Control system	Test system	Significantly different (α =.05)
Primary sludge*			
TS (g)	7.78	7.78	No
TVS (g)	6.59	6.59	No
Enzyme			
TS (g)	0.0228†	0.0228	No
Reactor effluent			
TS (g)	7.56	7.29	No
TVS (g)	6.34	6.04	No
Anaerobic digester effluent			
TS (g)	3.14	2.49	Yes
TVS (g)	1.92	1.47	Yes
Anaerobic digester gas production			
Gas L/g TVS input§	.650	.728	Yes
L**	4.12	4.62	Yes
g††	4.38	4.80	Yes
CH_4 L/g TVS input§	.439	.506	Yes
L**	2.78	3.21	Yes
g††	1.89	2.17	Yes
CO_2 L**	1.34	1.41	Yes
g††	2.49	2.63	Yes

* Data are reported on a 300 mL primary sludge input basis.
† Heat denatured enzyme.
§ Liters per gram TVS input.
** Measured volume.
†† Calculated from composition.

No significant differences (α = .05) were observed between the test and control system for total and volatile solids either entering or leaving the enzyme reactors. This result suggests that the increased liberation of reducing sugars from cellulose hydrolysis was not met with an immediate increase in biogas production. If this had been that case, a decrease in total solids leaving the test reactor and increase in solids lost as CO_2 would have occurred. This conclusion is supported by the data shown in Table 11. Volatile acids, potential metabolic products of reducing sugars, did not differ significantly (α = .05) between test and control reactors. The observed pH values also did not differ significantly (α = .05) between test and control reactors.

TABLE 11. VOLATILE ACIDS AND pH IN PRETREATMENT PROCESS REACTORS*

Analysis	Control	Test	Significantly different (α =.05)
Volatile acids (mg/L as HOAc)	4300	4772	No
pH	5.3	5.2	No

* Mean values of weekly analyses are shown.

Table 12 shows the results of cellulose analyses conducted on the raw primary sludge entering the reactors as well as the reactor effluents. The mean concentration of cellulose in the primary sludge over the course of this study was 19.6 percent of the total solids. The total solids effluents from the control and test reactors averaged 18.2 and 15.8 percent cellulose, respectively. These data indicate that the daily net cellulose hydrolysis resulting from addition of enzymes to the test reactor was 0.19 g from the original 1.53 g cellulose, or 12.4 percent. Comparison of this value to that predicted from Figure 16 at a loading of 2930 ppm for 16 hours shows that it fits well within the 95 percent confidence interval of 10 to 17.5 percent net cellulose hydrolysis expected.

Significant differences (α = .05) were observed in the gas production resulting from the test digester. The data in Table 10 show increases over the control of 12 and 15 percent for total gas and methane production per unit weight volatile solids input. Information available in the literature permits the stoichiometric calculation of gas production that can be expected from the metabolism of various feedstocks (25). Assuming an empirical formula of $C_6H_{10}O_5$ for cellulose, one can calculate yields from cellulose digestion for total gas and methane over a 15 day solids retention time of 0.73 L gas/g cellulose and 0.38 L CH_4/g cellulose, respectively. The increases due to enzyme addition shown in Table 10 are actually somewhat greater than that

TABLE 12. CELLULOSE ANALYSIS OF PRIMARY SLUDGE AND REACTOR EFFLUENTS IN THE CELLULOSE PRETREATMENT STUDY*

	Control system (g cellulose)/day	Test system (g cellulose)/day	Significantly different (α =.05)
Primary sludge	1.53	1.53	No
Reactor effluents	1.38	1.19	Yes
Wt cellulose hydrolyzed	0.15	0.34	Yes

* Mean values of weekly analyses are shown. The values represent weights of cellulose input and output on a daily basis.

predicted by stoichiometry. We suspect that this occurrence is related to the nature of the cellulose assay used. Since this assay measures cellulose bulk by filtration, it is sensitive only to changes in cellulose mass in terms of reducing sugars hydrolyzed from the cellulose molecules. Any subtle changes which would partially degrade the cellulose making it more amenable to conversion to biogas in the digester would therefore not be measured by this assay. In other words, it is suspected that this assay underestimates the amount of cellulose made available for bioconversion in the anaerobic digester. Table 13 presents mass and energy balance calculations for the solids and gas production data shown in Table 10. The mass balances are quite good (closure >93 percent) for both test and control systems. The energy balance calculations are somewhat less complete than the mass balances. It is felt that the input energy values may be somewhat low since heating values were computed for oven-dried sludge. Oven-dried sludge has been observed to be artificially low in certain fractions of the volatile solids such as oils and greases (9).

Figures 18 and 19 summarize the results of analyses for pH, volatile acids, and alkalinity in the test and control digesters. All values were within the normal ranges reported for digested sludge: pH 6.5 to 7.5, volatile acids 100 to 600 mg/L as HOAc, and alkalinity 2500-3500 mg/L as $CaCO_3$ (4). However, two important differences between the test and control systems should be noted. The alkalinity was consistently higher (α = .05) in the test system than the control, indicating it should have been more capable of buffering the organic acids produced by microbial conversion of cellulose-derived reducing sugars. However, it appears that this system was saturated near the end of the test run, as shown in Figure 19 when volatile acids increased dramatically to 425 mg/L as HOAc. During the same time interval the alkalinity began to decrease, apparently in response to this

TABLE 13. MASS AND ENERGY BALANCES FOR PRETREATMENT PROCESS LINE

Process stream	Control system Mass (g)	Control system Energy (Btu)	Test system Mass (g)	Test system Energy (Btu)
Input				
Primary sludge	7.78	128	7.78	128
Enzyme	.02	---	.02	---
Total	7.80	128	7.80	128
Output				
Biogas*	4.38	98	4.80	113
Digested sludge	3.14	47	2.49	37
Total	7.52	145	7.29	150
Balance $\frac{\Sigma output}{\Sigma input} \times 100$	96%	113%	93%	117%

* Heat content of methane assumed at 1000 Btu/ft^3.

increase in organic acids. This becomes more evident when one considers the impact on the bicarbonate alkalinity, which can be defined as follows: (25)

$$BA = TA - 0.71\ (VA)$$

Where: BA = bicarbonate alkalinity, mg/L as $CaCO_3$
TA = total alkalinity, mg/L as $CaCO_3$
VA = volatile acids, mg/L as HOAc

Computation using this formula shows that a decrease of about 20 percent in bicarbonate alkalinity accompanied the spike in volatile acids. These data suggest that perhaps an even greater response to cellulase enzyme addition could be expected if the buffering capacity of the digester could be increased.

Table 14 shows the mean values of the analyses performed as outlined in Table 9 for total Kjeldahl nitrogen (TKN) and total PO_4 - P in digested sludge for the test and control systems. There were no significant differences ($\alpha = .05$) between the test and the control system for these

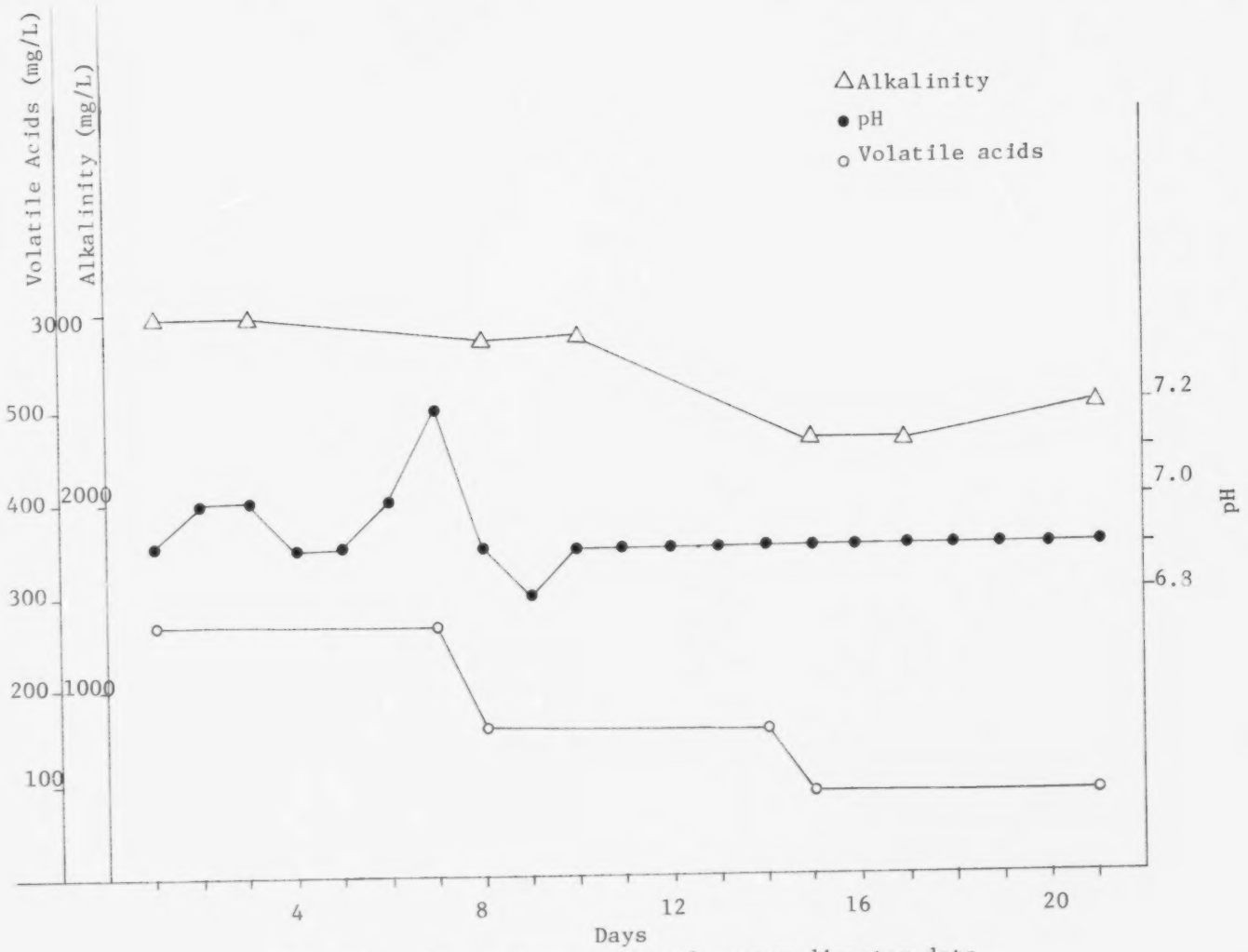


Figure 18. Pretreatment control system digester data.

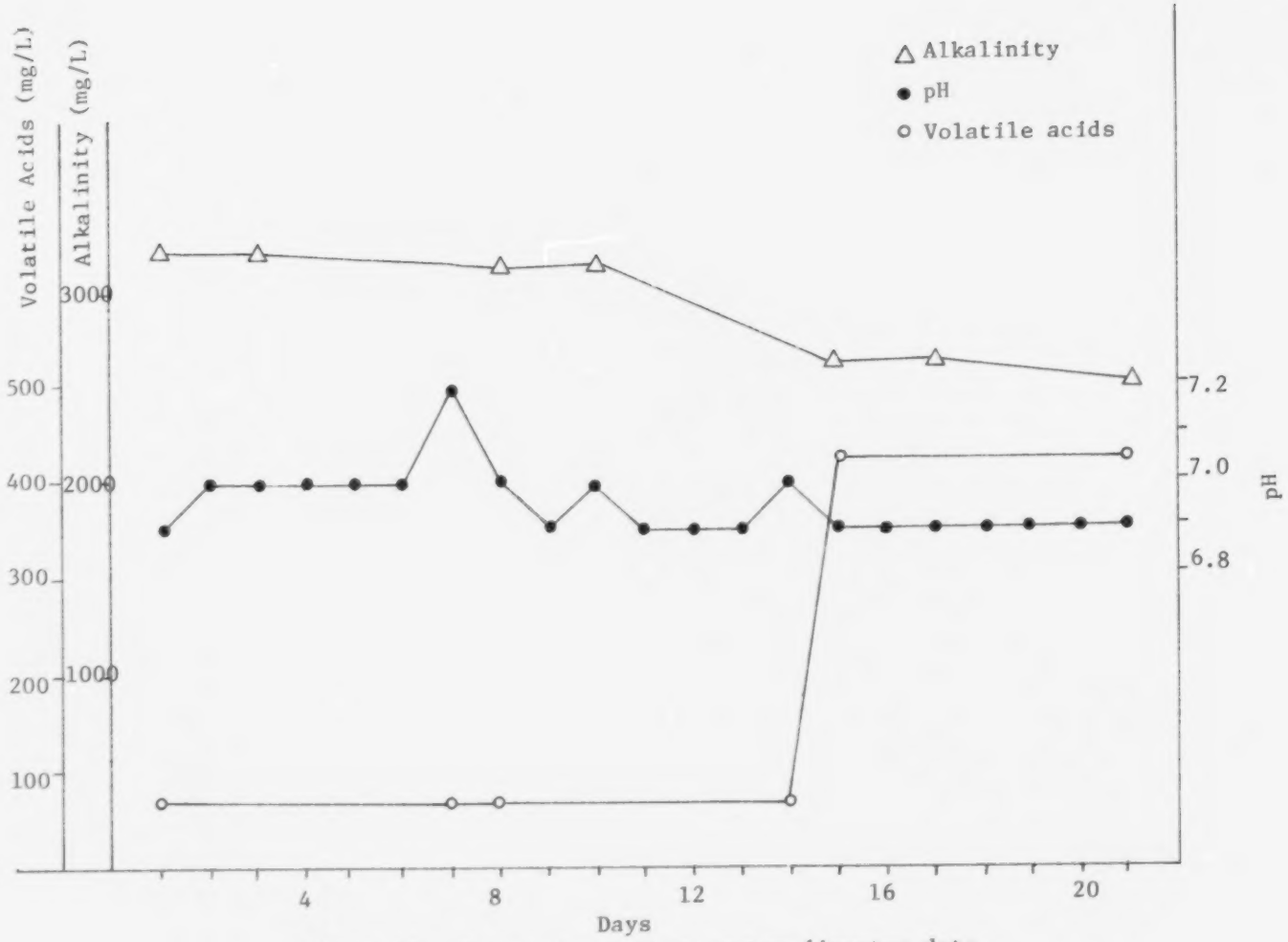


Figure 19. Pretreatment test system digester data.

TABLE 14. ANALYSIS OF TEST AND CONTROL CELLULASE PRETREATMENT SYSTEM DIGESTERS FOR TKN AND TOTAL P

Paramater	Control (mg/L)	Test (mg/L)
TKN (NH_3 as N)	1230	1150
Total P (PO_4 as P)	340	290

data. The TKN values represent a total solids nitrogen content of about 5 percent. This is well within the normal range for digested sludge, 1.6 to 6 percent of total solids (4). Both digesters showed ratios of nitrogen/phosphorus (N/P) on the order of 4:1. This is within the normal ratios expected for anaerobic metabolism, which is on the order of 5:1 (35,36).

Table 15 summarizes the results of additional analyses conducted on the digester effluents during the Pretreatment Process Study. Shown in this table are the average results of BOD analyses, filter yield, and specific resistance measurements, as well as heat content analyses of the filter cakes from the filter yield studies. The filtration studies were performed as described by Vesilind (37). The results of the BOD analyses showed that the test and control digesters were significantly different ($\alpha = .05$). However, since the test system was only 5 percent higher than the control, it is unlikely that this would result in major treatment problems. The increased BOD resulting from enzyme treatment suggests that a longer retention time might be warranted to digest the extra load of reducing sugars from cellulose hydrolysis.

The results of the filtration studies summarized in Table 15 are of use primarily as a means to compare the sludge characteristics of the test and control systems. The data cannot be used directly for design purposes since appropriate filter media were not available for these tests. Nevertheless, the data demonstrate that no significant differences ($\alpha = .05$) were observed in filter yield and specific resistance measurement between the test and control digesters. In addition, enzyme hydrolysis was found to have no significant effect ($\alpha = .05$) on the heating value of the filter cakes derived from the filter yield studies.

Table 16 summarizes the salient performance characteristics of the control and test systems during the Pretreatment Process Study. Also shown for comparison are results from a parallel single-stage digester operated during this study with no enzyme addition. The sludges and operating conditions employed for this system were identical to those of the test and control digesters. Perhaps the most obvious differences between the test,

TABLE 15. BOD, FILTRATION STUDIES, AND HEATING VALUE ANALYSIS FOR THE PRETREATMENT PROCESS STUDIES

Characteristic	Control system	Test system	Significantly different (α =.05)
Digested sludge (BOD [mg/L])	1387	1450	Yes
Filter yield (lb/ft^2/hr)	.45	.47	No
Specific resistance (m/kg)	19.4	17.6	No
Filter cake heating value (Btu/lb dry)	6840	6710	No

control, and comparison digesters can be seen in the volatile solids reduction and feed energy recovery. The use of active cellulase enzyme results in increases in volatile solids destruction of 56 percent and 10 percent over the comparison single-stage digester and control systems, respectively. Similar increases in feed energy recovery as methane are 57 percent and 14 percent. These differences are supported by data describing the biogas and methane production. The control system produced 0.60 standard m^3CH_4/kg VS destroyed, which is well within the range observed by other researchers for two-stage digestion of wastewater sludges (0.46 to 0.72 standard m^3CH_4/kg VS destroyed) (38). It is clear that the pretreatment concept offers a clear advantage over single-stage anaerobic sludge digestion in terms of both solids reduction and methane production. By comparing the test and control systems, however, it is also obvious most of the improvement gained by the process is due to going to a two-phase system by adding the pretreatment step (compare control to single-stage system).

POSTTREATMENT PROCESS CONCEPT

Three major experimental tasks were accomplished in evaluating the concept of cellulase enzyme posttreatment of anaerobic sludge. These included a preliminary study to characterize enzyme performance with digested sludge and an optimization study to examine the effects of time, temperature, and enzyme concentration. The final task was an in-depth process study of the cellulose posttreatment process concept.

Preliminary Study With Digested Sludge

The preliminary study with digested sludge was conducted using the same basic design and procedures as the preliminary study described earlier for the pretreatment process concept work. The major exception from this earlier study was that digested sludge was to be used as substrate. At the

time of testing, however, it was not possible to obtain a representative sample of sludge from the Glady Run plant because of severe digester stratification and scum layer formation. It was therefore necessary to reconstruct a test sample of "digested sludge" from scum layer and fluid removed from the bottom of the digester. This sample was made up using scum layer solids (TS = 13.1 percent) and diluting with digester liquid to a final TS = 4.9 percent.

TABLE 16. PERFORMANCE CHARACTERISTICS OF PRETREATMENT PROCESS SYSTEMS

	Control system	Test system	Single-stage system*
Temperature (°C)	35	35	35
Solids loading (g VS/L day)	1.5	1.5	1.4
Detention time (days)	15.7	15.7	15
Feed-TS (%)	2.59	2.59	2.45
Feed-VS (%)	84.7	84.7	81.4
Biogas production†			
Rate (vol/vol day)	.92	1.03	.64
Yield (standard m^3/kg VS added)	.63	.70	.48
(standard m^3/kg VS destroyed)	.89	.90	.97
CH_4 production§			
CH_4 (%)	67	69	70
Rate (vol/vol day)	.62	.71	.45
Yield (standard m^3/kg VS added)	.42	.49	.34
(standard m^3/kg VS destroyed)	.60	.63	.68
Efficiencies			
VS reduction (%)	71	78	50
Feed energy recovery as CH_4 (%)	77	88	56

* Computed from data on a parallel single-stage digester operated without primary sludge pretreatment.
† Single-stage gas production calculated by difference from solids mass balance data.
§ Single-stage CH_4 composition of 70 percent assumed.

The actual enzyme loadings applied in this study were 0, 73, 730, and 7300 by weight cellulose/sludge total solids basis. These loadings are nominally designated as 0, 10^2, 10^4, and 10^6 ppm in subsequent discussion. Cellobiase was used with celluclast at 1:10 weight ratio to maximize glucose production. All flasks were buffered at pH 4.8 with 0.1-M potassium hydrogen

phthalate buffer, nitrogen purged to remove dissolved gases, and incubated with a water gas trap at 35°C in a shaking incubator. Subsets of the flasks (triplicates for the control and each loading level) were removed at 24-, 48-, and 72-hr intervals.

Table 17 shows the results of the analysis of variance for the effects of incubation time and enzyme concentration on reducing sugar production, gas production, and cellulose hydrolysis. The analysis of reducing sugar flask concentrations showed significant effects (α =.05) due to incubation time, enzyme concentration, and their combination. Figure 20 shows the reducing sugar data obtained in this study. As in the comparable pretreatment study, reducing sugar production increased as a function of enzyme loading. In contrast to the pretreatment study, however, reducing sugar levels also increased as a function of time. In this case it appears that conditions were not appropriate in the digested sludge for rapid uptake of reducing sugars released by cellulose hydrolysis. This might be expected due to the negative effect on the normal bacterial populations of lowering the pH from 7 to 4.8.

TABLE 17. ANOVA RESULTS FOR PRELIMINARY STUDY WITH DIGESTED SLUDGE

Analysis	Source of variance	Sum of squares	Degrees of freedom	Mean square	Computed f	f Critical (α = .05)
Reducing sugar	time	118902.6	2	59,451.3	57.22	3.89
	conc	780685.8	3	260,228.6	250.47	3.49
	time/conc	76523.1	6	12,753.9	12.28	3.00
	error	12467.5	12	1,038.9		
		988549.0	23			
Gas ($\%CO_2$)	time	111.3	2	55.65	11.89	3.89
	conc	4394.5	3	1,464.8	312.9	3.49
	time/conc	84.7	6	14.12	3.02	3.00
	error	56.2	12	4.68		
			23			
Cellulose hydrolosis (% of initial hydrolyzed)	time	1294.72	2	647.36	25.5198	4.26
	conc	220.16	2	110.08	4.339	4.26
	time/conc	407.63	4	101.91	4.017	3.63
	error	229.83	9	25.367		
		2152.34	17			

Table 17 also presents the ANOVA results of gas analyses performed in this experiment. The gas data reported represent the percent replacement of nitrogen in the initial purge gas by carbon dioxide. Significant effects

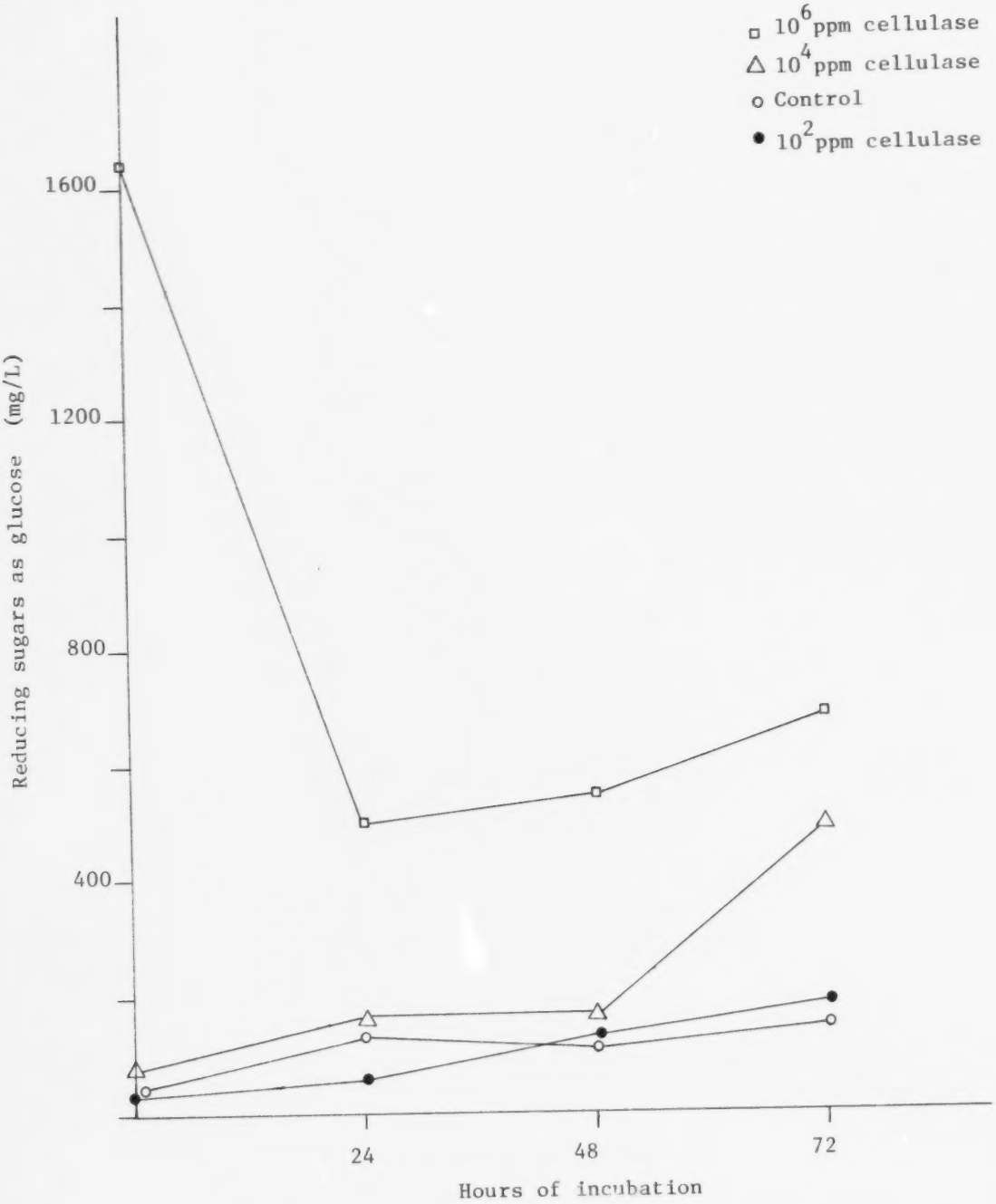


Figure 20. Reducing sugar production from cellulase enzyme treatment of digested sludge.

(α = .05) were observed in response to incubation time as well as enzyme concentration. Figure 21 shows the gas production data as a function of incubation time at various loading levels. At 24-hr incubation, the replacement by carbon dioxide rose to a plateau level which was generally maintained throughout the 72-hr incubation period. These data were quite similar to the data observed in the comparable pretreatment study.

The ANOVA for cellulose hydrolysis as a function of enzyme concentration is also reported in Table 17. Significant effects (α = .05) were observed in response to incubation time and enzyme concentration as well as their combination. Figure 22 shows the data plotted as the log of cellulose concentration (wt enzyme/wt sludge total solids basis) versus net cellulose hydrolysis. This relationship does not describe the data as well as in the pretreatment study. The data points at the lowest enzyme concentration appear to be artifically elevated, although no basis could be found to permit their rejection.

The largest computed f value for the ANOVA of this experiment corresponded to the effect of incubation time. This suggests the possibility that the fraction of cellulose remaining may be relatively resistant to enzyme hydrolysis and more highly dependent on time of reaction than on enzyme concentration. Figure 23 shows that the data appear to be more closely related to incubation time.

Optimization Study With Digested Sludge

The objective of the optimization study with cellulase application to digested sludge was to determine an optimum combination of incubation time, temperature, and enzyme concentration for maximum cellulose hydrolysis. A factorial design identical to that described previously in Figure 10 was applied. Two major deviations were made from the preliminary digested sludge experiment in order to maintain strict experimental control. First, the digested sludge was obtained from a laboratory-scale anaerobic digester operating on Glady Run primary sludge at 35°C with a 15-day retention time. Second, this sludge was preprocessed by passing through a 9.5-mm sieve to remove large inert particles which interfered with reproducibly dispensing the sludge.

The experiment was conducted by loading a series of 250-mL flasks with 100 mL of digested sludge from the laboratory-scale digester. The sludge (2.65 percent total solids, 4.95 percent cellulose) was incubated with cellulase at loadings of 0, 88, 880, and 8800 ppm by weight cellulase/sludge total solids. Cellobiase was added with the cellulase in a 1:10 weight ratio. Flasks were buffered at pH 4.8 with 0.1-M KHP. The flasks were purged with nitrogen to remove dissolved gases and incubated with a water gas trap in a Lab-Line Orbit Environ Shaker at 3.4 Hz with a 2-cm displacement. Subsets of the flasks (triplicate for the control and each loading level) were incubated at 35°, 40°, and 50°C for 1, 8, and 16 hours. Table 18 shows the results of the ANOVA performed on the experimental data with cellulose

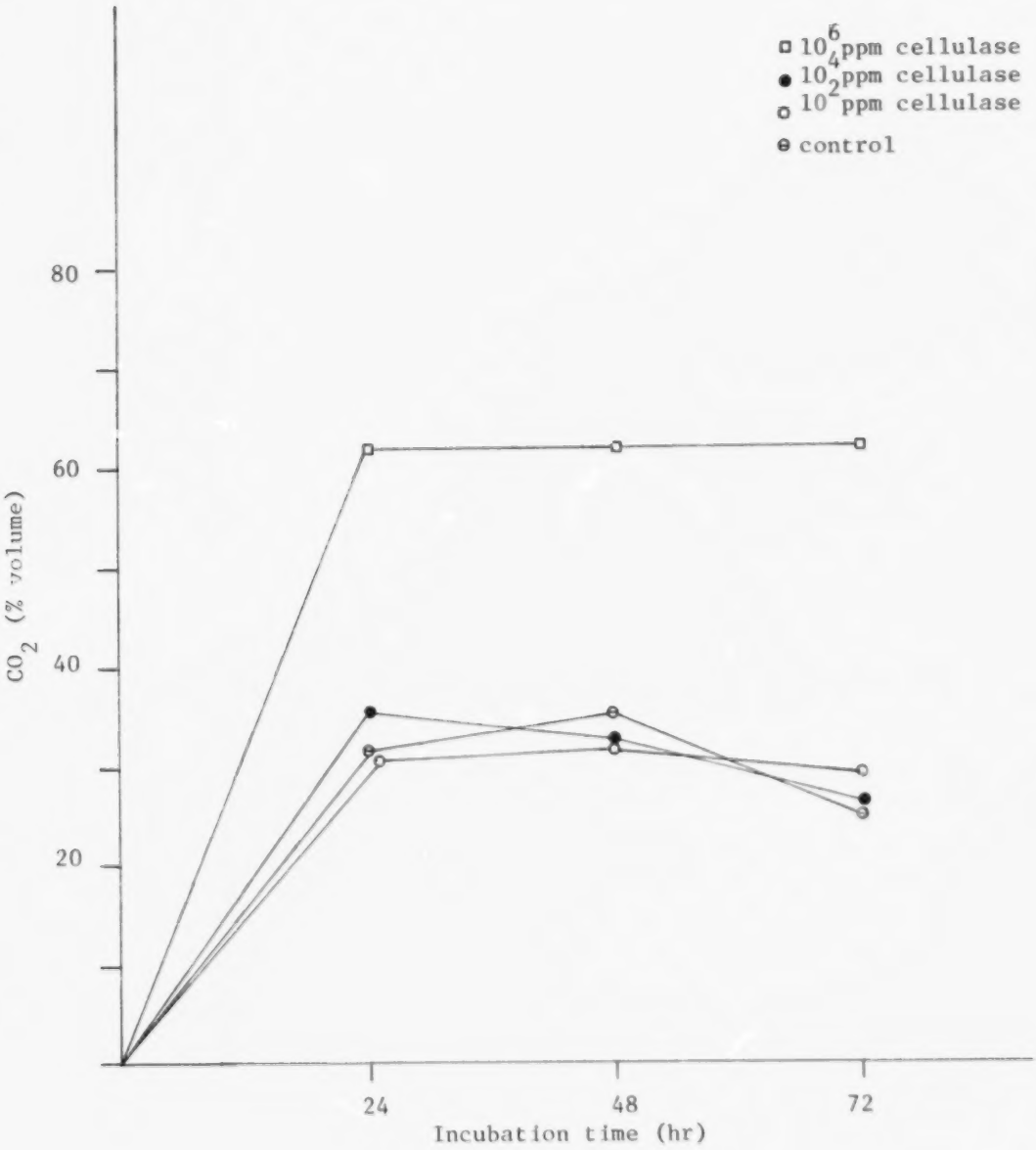


Figure 21. Carbon dioxide concentration resulting from cellulase treatment of digested sludge.

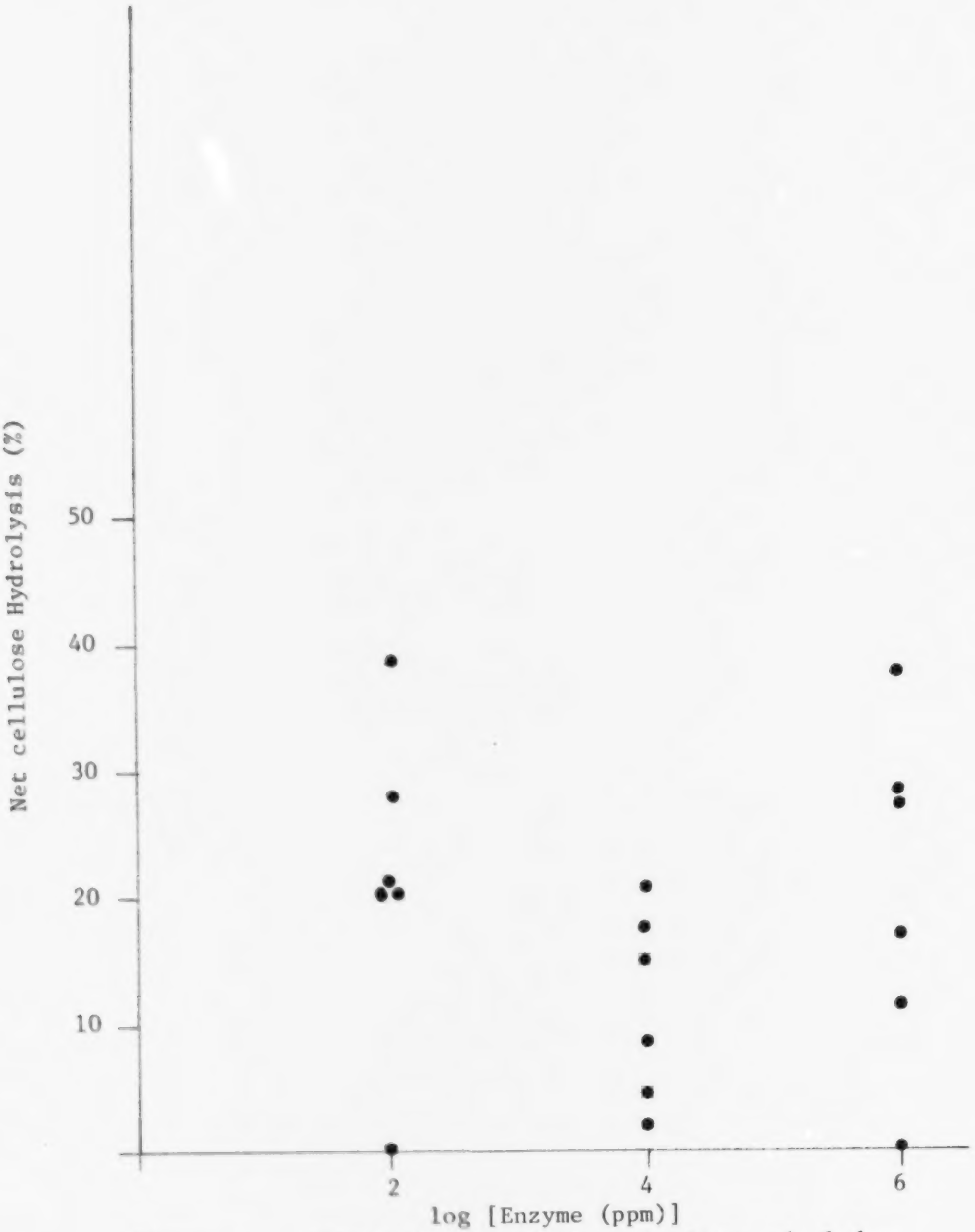


Figure 22. Hydrolysis of cellulose in digested sludge.

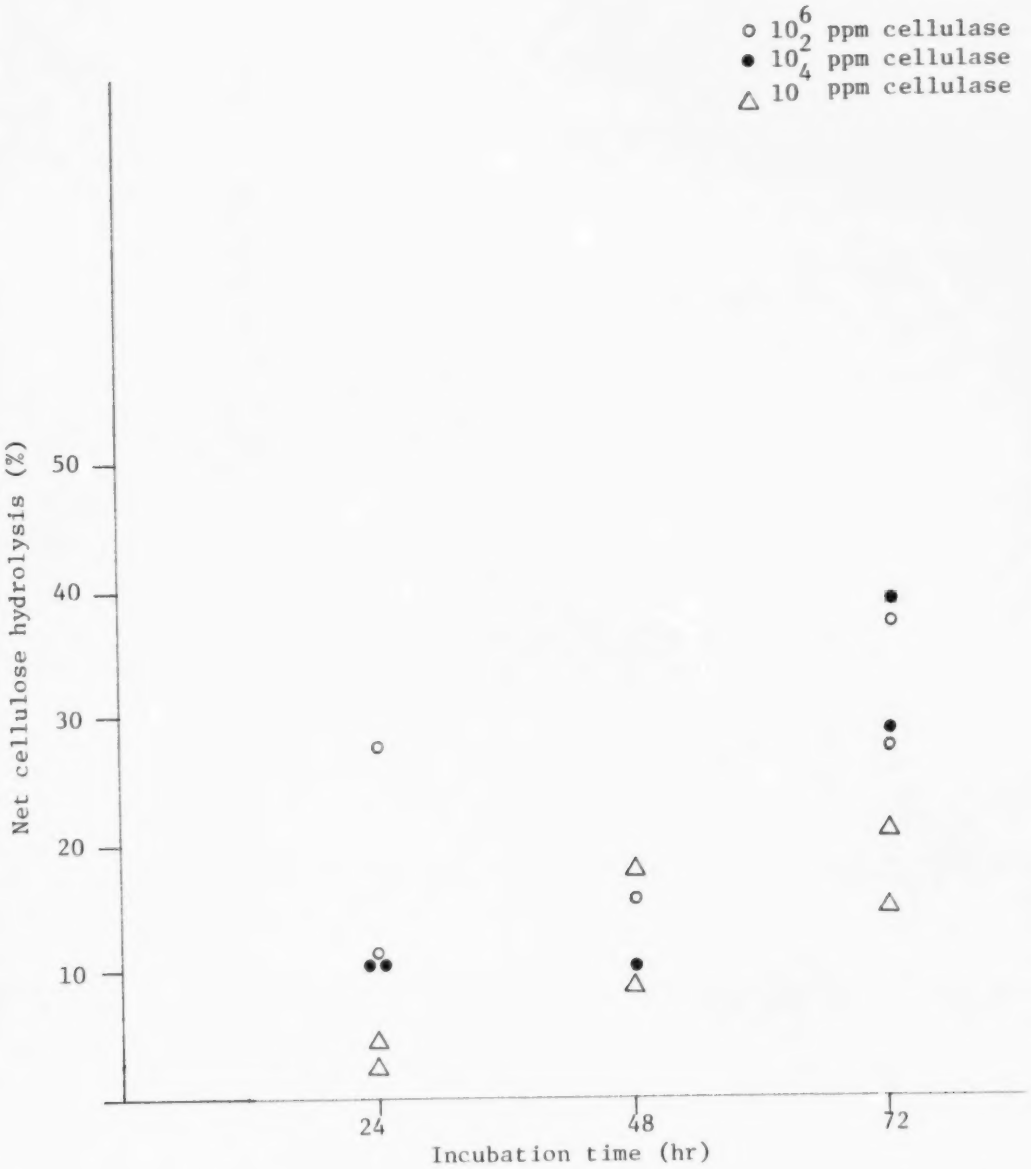


Figure 23. Cellulose hydrolysis in digester sludge as a function of time.

hydrolysis as the master variable responsive to time, temperature, and enzyme concentration. Using an $\alpha = .05$ as a critical value, none of the single factors or combinations showed a significant effect on cellulose hydrolysis. At an $\alpha = 0.1$ only incubation time showed a clear effect on cellulose hydrolysis. No further analyses of data were performed after reviewing the results of the ANOVA.

TABLE 18. POSTTREATMENT OPTIMIZATION STUDY ANOVA RESULTS

Analysis	Source of variance	Sum of squares	Degrees of freedom	Mean square	Computed f	f Critical ($\alpha = .05$)
	Temp	.1091E-02	2	.5455E-03	1.58	.2470
	Conc	.2105E-02	3	.7015E-03	2.03	.1641
Weight	Temp × conc	.4875E-02	6	.8125E-03	2.35	.0984
cellulose	Time	.2202E-02	2	.1101E-02	3.18	.7801E-01
hydrolyzed	Temp × time	.4322E-03	4	.1080E-03	.31	.8645
	Conc × time	.2158E-02	6	.3597E-03	1.04	.4475
	Time × temp × conc	.4156E-02	12	.3463E-03		
	(error)					

Levels of test variables:

Temperature	50°, 40°, 35°C
Enzyme concentration	0, 88, 880, 8800 (wt enzyme/wt sludge total solids)
Time	1, 8, 16 hr

A critical analysis of the optimization study data suggested several reasons why application of cellulase to digested sludge was not as successful as anticipated. One major potential problem relates to the physiological state of the digested sludge. It has already been shown in the preliminary digested sludge experiment that reducing sugars accumulated over time. This information suggests that product inhibition of the enzyme by liberated glucose may have occurred. As noted earlier, the cellulose remaining after digestion may also be relatively resistent to further degradation. In addition, it is possible that critical factors such as volatile acid or alkalinity levels may have been upset by the use of enzyme. Before abandoning this concept entirely, it was decided to pursue a carefully controlled Posttreatment Process Study in order to evaluate this process concept in more depth.

Process Study

Figure 24 provides a schematic illustration of the two laboratory systems employed to evaluate the cellulase posttreatment process concept over an extended time interval. The control system differed from the test system in that heat-denatured enzyme was added to the control, while the test system received active enzyme. A separate laboratory-scale feed digester (4.5-L volume, 15-day retention time, 35°C) was operated to provide substrate for the posttreatment process. This digester was operated on primary sludge from the Glady Run plant; the same primary sludge was also used to provide a nutrient supplement to the posttreatment digesters. It should be noted that a normal configuration for this process concept would involve recycling of reactor effluent to the feed digester. A secondary digester (the test or control digester in Figure 24) was used in the study to allow separation of gas production and solids destruction data.

The control and test reactors consisted of 500-mL flasks incubated in a batch mode for 16 hours at 35°C in a Lab-Line® Orbit Environ-Shaker at 3.4 Hz with 2-cm displacement. The digesters, shown previously in Figure 2, were 6-L glass flasks equipped with gas collection devices and maintained at 35°C for a 7.5-day hydraulic retention time.

The process lines were operated as semicontinuous flow processes. The duration of this study was 48 days. Table 19 summarizes the daily volume flows through the systems. The actual reactor volumes were slightly higher than shown to provide aliquots for the required analyses. The primary sludge was routinely filtered through a 9.5-mm sieve to remove large inert particles. Table 20 summarizes characteristics of the enzyme-treated digested sludge and primary sludge fed to the test and control system digesters. The digesters were fed and gas production recorded once each day at approximately 1200 hours. The digesters were thoroughly mixed by shaking three times each day at approximately 0800, 1200, and 1700 hours.

The experimental design employed for this process study was basically identical to that of the Pretreatment Process Study. The objective was to determine whether the test system performed better than the control. The same test protocol was followed: equivalent performance was required before and after testing for at least two hydraulic retention times (15 days). All data taken during the test phase were analyzed on a daily or weekly basis utilizing a t-test for paired observations with an $\alpha = .05$. This approach was taken to minimize the effects of day-to-day variation on systems performance. During the study, enzyme was added to the systems at a mean concentration of 2710 ppm by weight (0.27 percent by weight) to parallel the conditions of the Pretreatment Process Study. Table 21 summarizes the measurements performed during the test.

Table 21 also summarizes the mean values for solids and gas data obtained during the Posttreatment Process Study. All data are included in the Appendix. Also included in this table is a statement regarding the presence

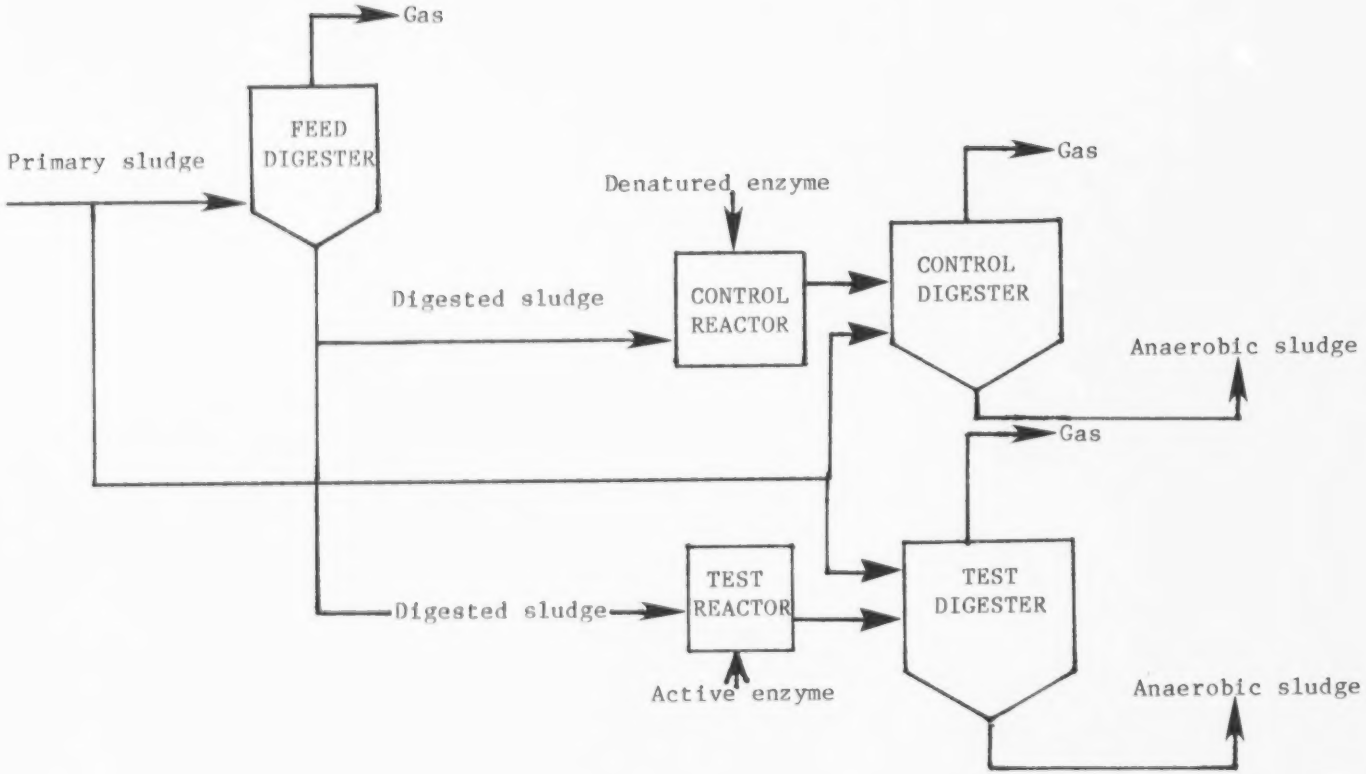


Figure 24. Control and test systems for Cellulase Posttreatment Process Study.

of statistically significant (α = .05) differences between the control and test systems data. These data were determined by computing daily differences between the systems and applying a paired t-test (7).

TABLE 19. DAILY VOLUME FLOWS FOR CELLULASE POSTTREATMENT PROCESS STUDY

Stream	Control system (mL)	Test system (mL)
Primary sludge to feed digester*	300	300
Digested sludge to reactors	150	150
Enzyme to reactors	10†	10
Reactor effluent to digesters§	150	150
Primary sludge to digesters	150	150
Digester effluents	300	300

* Feed digester maintained at 4500 mL with a 15-day hydraulic retention time.
† Heat denatured enzyme.
§ 10 mL portions of reactors removed for analysis prior to digester feeding. Digesters maintained at 2250 mL with a 7.5-day hydraulic retention time.

The feed digester performed as a typical high rate system, destroying approximately 50 percent of the input volatile solids. The gas production for this system, shown in Table 22, was estimated from a solids mass balance and the assumption of 70-mole percent methane in the biogas. The sludge effluent from the feed digester was split into two equal portions daily to serve as substrate for the test and control reactors.

The effluents from the test and control reactors were supplemented with primary sludge before addition to the test and control digesters. Although cellulose analyses were not performed on the input streams to these digesters, analysis of digester effluents showed a significant difference (α = .05) in cellulose concentration. The control and test digesters showed that 9.5 and 8.8 percent of the total solids were identified as cellulose. These data provide evidence for enhanced digestion of cellulose through the use of active cellulase enzyme.

TABLE 20. PRIMARY AND DIGESTED SLUDGE FEED CHARACTERISTICS FOR POSTTREATMENT PROCESS STUDY

Characteristic	Mean value
Digested sludge feed	
TS (percent)	1.77
VS (percent of TS)	63.0
pH	6.8
Raw primary feed	
TS (percent)*	2.50
VS (percent of TS)	75.7
pH	5.5

* Adjusted by dilution with tap water.

Significant differences (α =.05) were also observed in the gas production and solids destruction from the test and control digesters. In contrast to the pretreatment study described earlier, however, the test system showed a significantly decreased performance in comparision to the control. The data in Table 22 show that total gas and methane per unit weight volatile solids input from the test system represented only 55 and 41 percent of the control system values. The total and volatile solids content of the test system digester were also significantly higher than control values.

Table 23 presents mass and energy balance calculations for the solids and gas data reported in Table 22. Mass balances for both the test and control systems are excellent (closure >95 percent). As in the pretreatment study, calculated energy outputs exceed inputs. This situation is probably due to an underestimate of feedstock heat content.

Figures 25 and 26 summarize the analytical results of pH, volatile acids, and alkalinity in the test and control digester. They also offer one possible explanation for the decreased performance of the test system despite an overall increase in cellulose hydrolysis. Evaluating the data with a paired t-test approach indicates that volatile acids were significantly higher (α = .05) in the test system than the control, with test period averages of 920 and 380 mg acetic acid/L, respectively. This trend is especially pronounced on examination of the Day 24 data, where test system volatile acids exceeded control data by a factor of 2.4. Subsequent to this date, a significant drop in test system digester pH data was also observed. Analysis of alkalinity data at Day 24 shows that test and control systems were 1750 and 2150 mg/L $CaCO_3$, respectively. Calculations of bicarbonate alkalinities as described earlier in this report show that test and control systems registered 290 and 1550 mg/L $CaCO_3$ respectively. This dramatic decrease in test system

TABLE 21. ANALYSIS SCHEDULE FOR THE CELLULASE POSTTREATMENT PROCESS STUDY

		TEST SYSTEM							CONTROL SYSTEM					
Analysis	Xenia primary sludge	Feed digester out	Reactor in	Reactor out	Digester in	Digester out	Filter cake	Filtrate	Reactor in	Reactor out	Digester in	Digester out	Filter cake	Filtrate
TS	D	D		MWF		MWF	C			MWF		MWF	C	
VS	D	D		MWF		MWF	C			MWF		MWF	C	
pH	D	D		D		D				D		D		
Gas volume						D						D		
CH_4 percent						D						D		C
CO_2 percent						D						D		
Volume	D	D	D	D	D	D		C	D	D	D	D		
Weight	D	D	D	D	D	D	C		D	D	D	D	C	
Enzyme weight			D						D					
Volatile acids				C		C				C		C		
Alkalinity						C						C		
TKN						C						C		
Total P						C						C		
BOD						CX						CX		
Btu/lb	C					C	C					C	C	
Filter yield						C						C		
Specific resistence						C						C		
Cellulose	C					C				C				

D = daily
MWF = Monday, Wednesday, Friday
C = weekly composite
CX = weekly composite, analysis performed by Xenia

TABLE 22. SOLIDS AND GAS PRODUCTION DATA FOR THE POSTTREATMENT PROCESS STUDY (Daily Basis)

Process stream	Control system	Test system	Significantly different (a = .05)
Primary sludge to feed digester*			
TS (g)	4.49	4.49	No
TVS (g)	3.31	3.31	No
Digested sludge from feed digester			
TS (g)	2.65	2.65	No
TVS (g)	1.67	1.67	No
Gas from feed digester*†			
total L§	1.60	1.60	No
total g**	1.64	1.64	No
CH_4 L§	1.13	1.13	No
g**	.76	.76	No
CO_2 L§	.48	.48	No
g**	.89	.89	No
Enzyme			
TS (g)	.0072††	.0072	No
Reactor effluent			
TS (g)	2.65	2.63	No
TVS (g)	1.67	1.63	No
Primary sludge to digester			
TS (g)	3.75	3.75	No
TVS (g)	2.84	2.84	No
Digester effluent			
TS (g)	4.85	5.16	Yes
TVS (g)	3.09	3.42	Yes
Gas from digester			
Total L/g TVS input§§	.341	.187	Yes
L§	1.54	0.84	Yes
g**	1.57	1.04	Yes
CH_4 L/g TVS input§§	.241	.099	Yes
L§	1.09	.45	Yes
g**	.73	.30	Yes
CO_2 L§	.45	.40	Yes
g**	.84	.74	Yes

* Data for feed digester are reported on 150-mL basis to match input to reactors.
† Gas data calculated assuming 70-mole percent CH_4 in biogas.
§ Measured volume.
** Calculated from composition.
†† Heat denatured enzyme.
§§ Liters per gram TVS input.

TABLE 23. MASS AND ENERGY BALANCES FOR CELLULASE POSTTREATMENT PROCESS STUDY

Process stream	Control system		Test system	
	Mass (g)	Energy (Btu)	Mass (g)	Energy (Btu)
Input				
Primary sludge to:				
feed digester*	4.49	74	4.49	74
test and control digesters	3.75	62	3.75	62
Enzyme	0.01	--	0.01	--
TOTAL	8.25	136	8.25	136
Output				
Biogas† from:				
feed digester§	1.64	40	1.64	40
test and control digester	1.57	38	1.04	16
Digested sludge	4.85	79	5.16	84
TOTAL	8.06	157	7.84	140
Balance $\frac{\Sigma \text{output}}{\Sigma \text{input}} \times 100$	98%	115%	95%	103%

* Based on 150-mL volume distribution to each reactor.
† Heat content of methane assumed at 1000 Btu/ft^3.
§ Estimated from mass balance around feed digester and assumption of 70 percent methane in the biogas.

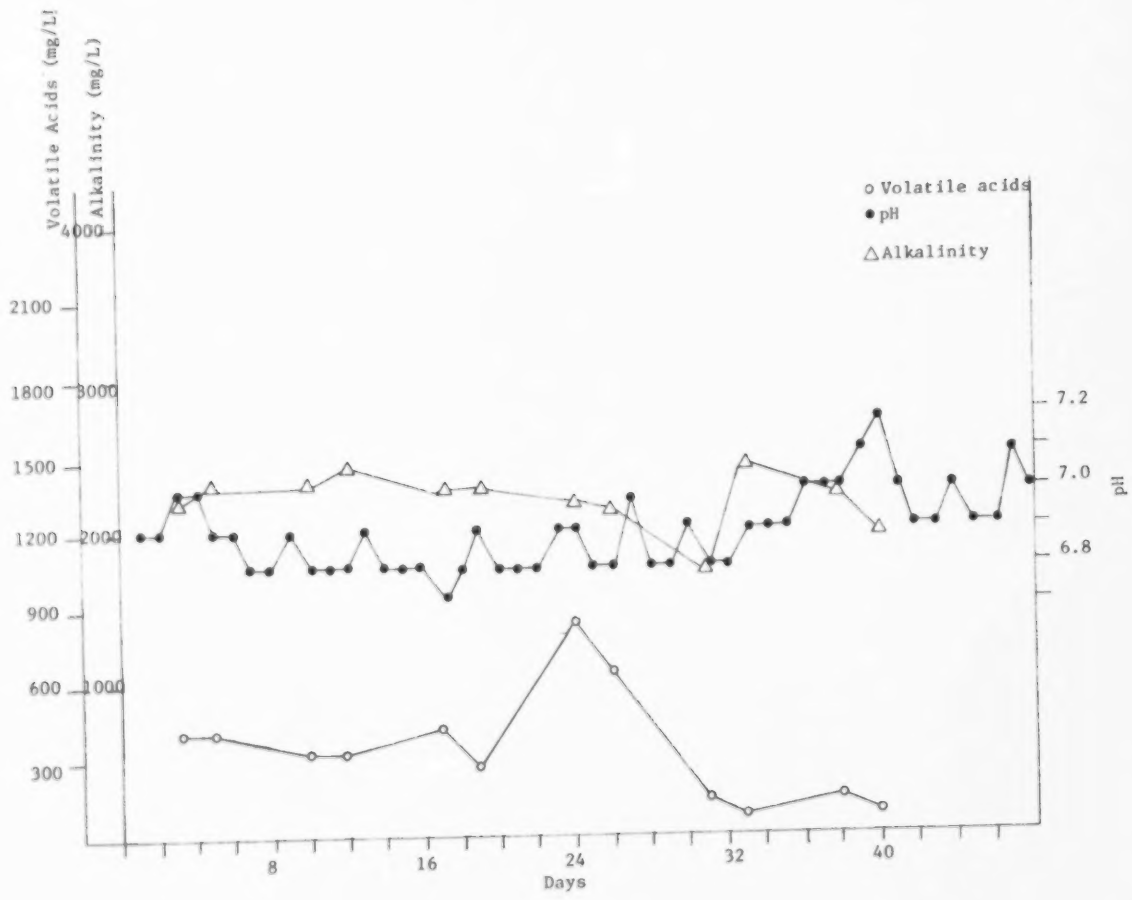


Figure 25. Posttreatment control system digester data.

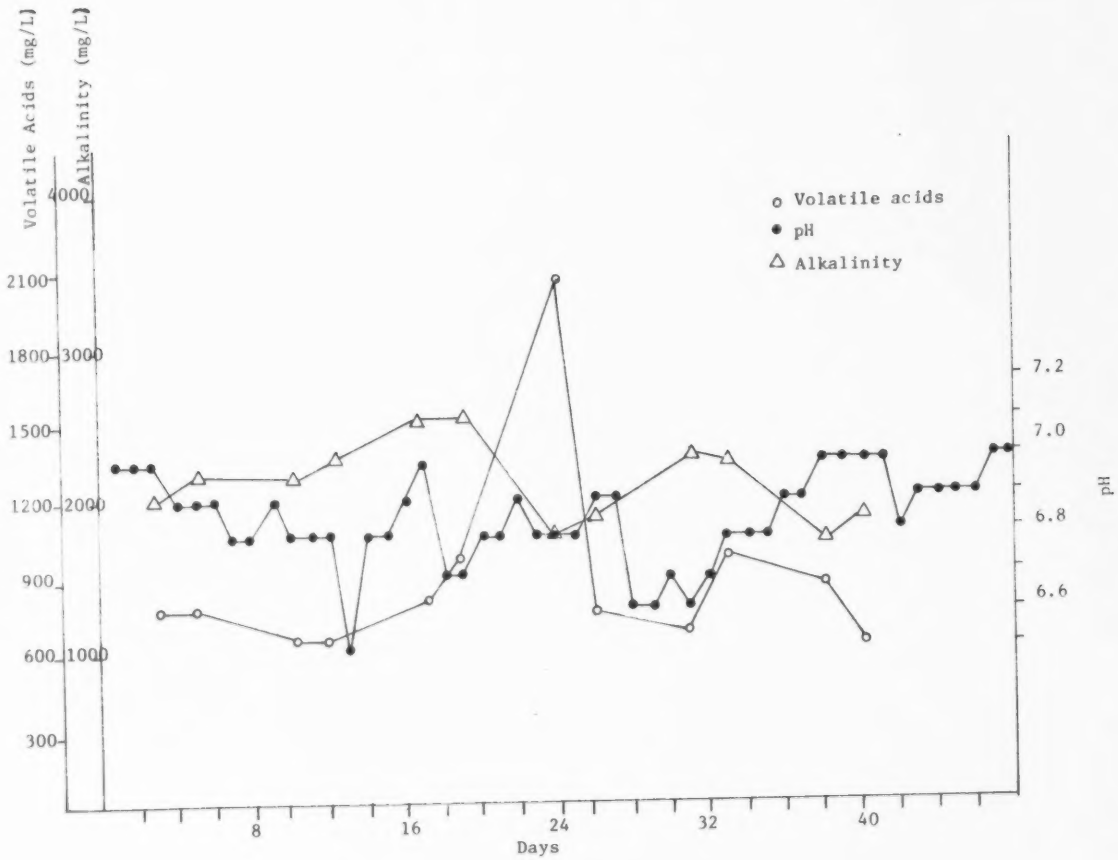


Figure 26. Posttreatment test system digester data.

alkalinity represents a major upset in digester health which could logically be expected to reduce gas production and solids destruction. The ultimate cause for this upset situation could have resulted from increased cellulase hydrolysis producing an excess of reduction sugars in the test system reactor. Given the short retention time in the digester (7.5 days), this loading of sugars may have accumulated as volatile acids and not been further metabolized to biogas.

Table 24 shows the results of analyses for TKN and total P in the test and control digesters. No significant differences ($\alpha = .05$) were observed between test and control data. The TKN values represent a total solids nitrogen content of 4 percent. This value is less than the pretreatment study, yet still within the normal digester range (1.6 to 6 percent of total solids) (4). The N/P ratios were again in the range of 3 to 4:1, within the normal range of 5:1 (35, 36).

TABLE 24. ANALYSIS OF TEST AND CONTROL CELLULASE POSTTREATMENT DIGESTERS FOR TKN AND TOTAL P

Parameter	Control (mg/L)	Test (mg/L)
TKN (NH_3 as N)	780	690
Total P (PO_4 as P)	170	200

Table 25 summarizes the results of BOD analyses, filter yield, and specific resistance measurements, as well as heat content analyses of the filter cakes from the filter yield studies. The filtration studies were performed as described by Vesilind (37). The results of BOD analyses showed that the test and the control systems were significantly different ($\alpha = .05$). The increased BOD of the test system relates well with the overall decreased system performance and could be expected to result in treatment problems. The filter yield was also significantly less ($\alpha = .05$) in the test system, supporting the overall decrease in system performance. As stated earlier in this report, the filtration data reported are suitable for comparative purposes only and are not appropriate as design parameters.

Table 26 summarizes the important performance characteristics of the control and the test systems during the Posttreatment Process Study. The feed digester is included as a separate line entry. It is clear that the feed digester operated essentially identically to the single-phase digester reported previously in Figure 16. This system produced 0.98 standard m^3 gas/kg VS destroyed, which is well within the range observed by other

TABLE 25. BOD, FILTRATION STUDIES, AND HEATING VALUE ANALYSES FOR THE POSTTREATMENT PROCESS STUDY

Characteristic	Control system	Test system	Significantly different (α=.05)
Digested sludge BOD (mg/L)	2566	4938	yes
Filter yield (lb/ft^2/hr)	.54	.50	yes
Specific resistance (m/kg)	20.7	25.7	no
Filter cake heating value (Btu/lb/dry)	7408	7748	no

researchers for wastewater sludge digestion (0.75 to 1.12 standard m^3 gas/kg VS destroyed) (38). The performance of the control digester was also within the acceptance range for total gas production; however, the test digester was significantly lower at 0.59 standard m^3 gas/kg VS destroyed. Methane production rates paralleled this trend. It is interesting to note that although the control system achieved an overall volatile solids reduction of only 31 percent, this reduction would account for a 50 percent removal of primary sludge from this vessel if one assumes the digester sludge feed was not further degraded. This point is particularly interesting in view of the fact that the retention time in the control digester was only 7.5 days. Although the test system did not perform as well as the control in any of the performance calculations shown in Table 26, previous discussion suggests that implementing methods to control volatile acids might significantly improve its performance. In this manner the improved cellulose hydrolysis in the test system might be expected to yield improved gas production and solids destruction.

SIMULTANEOUS TREATMENT PROCESS CONCEPT

The initial concept of simultaneous cellulase enzyme treatment and anaerobic digestion involved direct addition of enzyme to the digestion vessel. In theory this concept was considered highly attractive since there would be no requirement for additional vessels or extensive support equipment. An experimental protocol to examine this concept was initially designed to parallel the pre- and postreatment process studies. However, in the course of this program it was discovered that the pH optimum of the test enzyme was not suitable for use in an anaerobic digester. This point was experimentally verified before proceeding with a full-scale investigation of simultaneous treatment.

TABLE 26. PERFORMANCE CHARACTERISTICS OF POSTTREATMENT PROCESS SYSTEMS

	Feed digester	Control digester	Test digester
Temperature (°C)	35	35	35
Solids loading (GVS/L/day)	1.47	2.00	2.00
Detention time (days)	15	7.5	7.5
Feed TS (%)	2.99	2.13	2.13
Feed VS (%)	73.7	70.5	70.5
Biogas production*			
Rate (vol/vol day)	.71	.68	.37
Yield (standard m^3/kg VS added)	.48	.34	.19
(standard m^3/kg VS destroyed)	.98	1.08	.59
CH_4 production†			
CH_4 (%)	70.0	70.8	53.6
Rate (vol/vol/day)	.50	.48	.20
Yield (standard m^3/kg VS added)	.34	.24	.10
(standard m^3/kg VS destroyed)	.69	.77	.32
Efficiencies			
VS reduction (%)	50	31	24
Feed energy recovery as CH_4 (%)	54	37	15

* Single-phase gas production calculated by difference from solids mass.
† Single-phase CH_4 composition of 70 percent assumed.

In this experiment, a preliminary investigation of cellulase enzyme application to digested sludge was conducted. Digested sludge from the parallel single-phase digester described earlier was used as substrate for this experiment. The sludge was prescreened through a 9.5-mm sieve before use. The sludge total solids, volatile solids, and pH were measured at 1.2 percent, 40.6 percent, and 7.1, respectively. No buffer for pH control was included. A series of flasks containing 100-mL sludge were set up for 120-hr incubation at 35°C. Enzyme loadings employed were 0, 483, 4830, and 48,300 ppm by weight enzyme/sludge total solids. Incubation was conducted in a Lab-Line® Orbit Environ-Shaker at 3.4 Hz with a 2-cm displacement with triplicates for each loading level.

Table 27 illustrates the results of this experiment. All flasks showed a cellulose content significantly lower ($\alpha = .05$) than the amount initially present. However, there were no significant differences ($\alpha = .05$) between

TABLE 27. CELLULOSE ANALYSIS OF DIGESTED SLUDGE FOLLOWING 120-HR INCUBATION AT pH 7 AND 35°C*

Enzyme loading (ppm)	Weight cellulose remaining (g)	Standard deviation (s)
0	.0483	.0038
483	.0548	.0006
4830	.0546	.0011
48300	.0489	.0006

* Initial analysis before incubation showed .0927 ± .0065-g cellulose.

any of the incubated flasks. These data indicated that cellulose hydrolysis was not enhanced by enzyme application to digested sludge, presumably because of the inappropriate pH conditions.

SECTION 5

LIPASE ENZYME PROGRAM

SIGNIFICANCE OF PROBLEM

It was noted early in the program that oil and grease represent a major operational problem for anaerobic digesters. Our initial attempts to perform testing utilizing digested sludge from Glady Run were frustrated by severe stratification and scum layer formation in the Xenia digester. An analysis of samples from the scum layer showed that it was composed of 13.1 percent total solids distributed as 44.9 percent oil and grease, 9.1 percent cellulose, 32.7 percent inerts, and 13.3 percent unclassified volatiles.

In view of this high concentration of oil and grease, coupled with the limited potential for success of the simultaneous treatment concept with cellulase, the EPA approved a request to modify the program scope to include evaluation of lipase enzyme augmentation to enhance oil and grease destruction in the anaerobic digester. This modification was structured to provide information parallel to the cellulase enzyme program. The first task included development and verification of a method to assay the hydrolysis of oils and greases. The second task involved an optimization study to determine appropriate levels of enzyme addition and incubation time for optimum hydrolysis. The final task consisted of operating a process study utilizing lipase enzyme within the digester. All work conducted in this segment of the program utilized Novozyme 225, a lipase enzyme product from Novo Laboratories, Inc. previously described in Table 3.

ENZYME CHARACTERIZATION WITH ARTIFICIAL SUBSTRATE

Although the oil and grease fraction of primary sludge was the primary substrate used in the lipase program, initial experiments were conducted with the lipase enzyme utilizing soybean oil as an artificial substrate. The objective of this work was to expand upon manufacturers' information regarding the enzyme and to develop an assay to evaluate the extent of enzymatic hydrolysis. Table 28 shows the experimental conditions employed in these experiments. A high enzyme loading was employed to ensure easily interpreted results.

The initial experiment examined the following matrix of test conditions: no oil/no enzyme, oil/no enzyme, and oil/enzyme. At the conclusion of the incubation period, aliquots of the reaction flasks were titrated with 0.1-M NaOH to determine the amount of fatty acids liberated. Figure 27 illustrates the pH titration curves so obtained. By comparing the inflection

TABLE 28. EXPERIMENTAL CONDITIONS FOR INITIAL LIPASE STUDY

Reaction temperature:	40°C
Reaction time:	24 hr in a Lab-Line® Orbit Environ-Shaker at 3.4 Hz with 2-cm displacement
Reaction pH:	7.0 with 0.1-M potassium phosphate buffer
Reactor contents: (100 ml volume basis)	20-mL emulsion reagent* with 0.58-g soybean oil 80-mL buffer 5-mL anaerobic digester supernatant 10-mL enzyme solution† or buffer

* Emulsion reagent composed of:
7.35g $CaCl_2$ $2H_2O$
2.93g NaCl
20.00-g gum arabic
8-mL 0.1 N NaOH
diluted to 1 liter with deionized water

† Enzyme loading at 86,000 ppm (8.6 wt percent) enzyme/oil basis.

points of the curves for no oil/no enzyme and oil/no enzyme, it can be determined that 0.15 mL of 0.1 N NaOH were required to titrate the fatty acid liberated from oil without enzyme, equivalent to the release of 1.48×10^{-4} mole fatty acid/g oil. The amount of fatty acid liberated by the addition of enzyme can be determined from the difference between the inflection points of the oil/enzyme and oil/no enzyme curves on Figure 27. This value, 1.35 mL, correspond to the release of 1.33×10^{-3} mole fatty acid/g oil. In order to relate this information to extent of hydrolysis, it was necessary to first compute the total titratable fatty acids present in the soybean oil. This was accomplished by saponifying the oil in 0.5-M KOH and titrating the remaining KOH with 0.5 M HCl according to standard procedures (39). Figure 28 illustrates the results of the saponification experiment. The saponification experiment was conducted in triplicate and the mean value used for all sequent calculations. These data indicate a mean saponification value of 208 mg KOH/g soybean oil, equivalent to 3.55×10^{-3} mole fatty acid/g oil, which compares well with the reference value of 191 mg KOH/g (40).

Analysis of the data shown in Figure 27 and 28 indicated that 4 percent of the available oil was hydrolyzed without the addition of enzyme, while an additional 33 percent was hydrolyzed by employing enzyme augmentation. The addition of enzyme therefore resulted in nearly a 9-fold increase in the

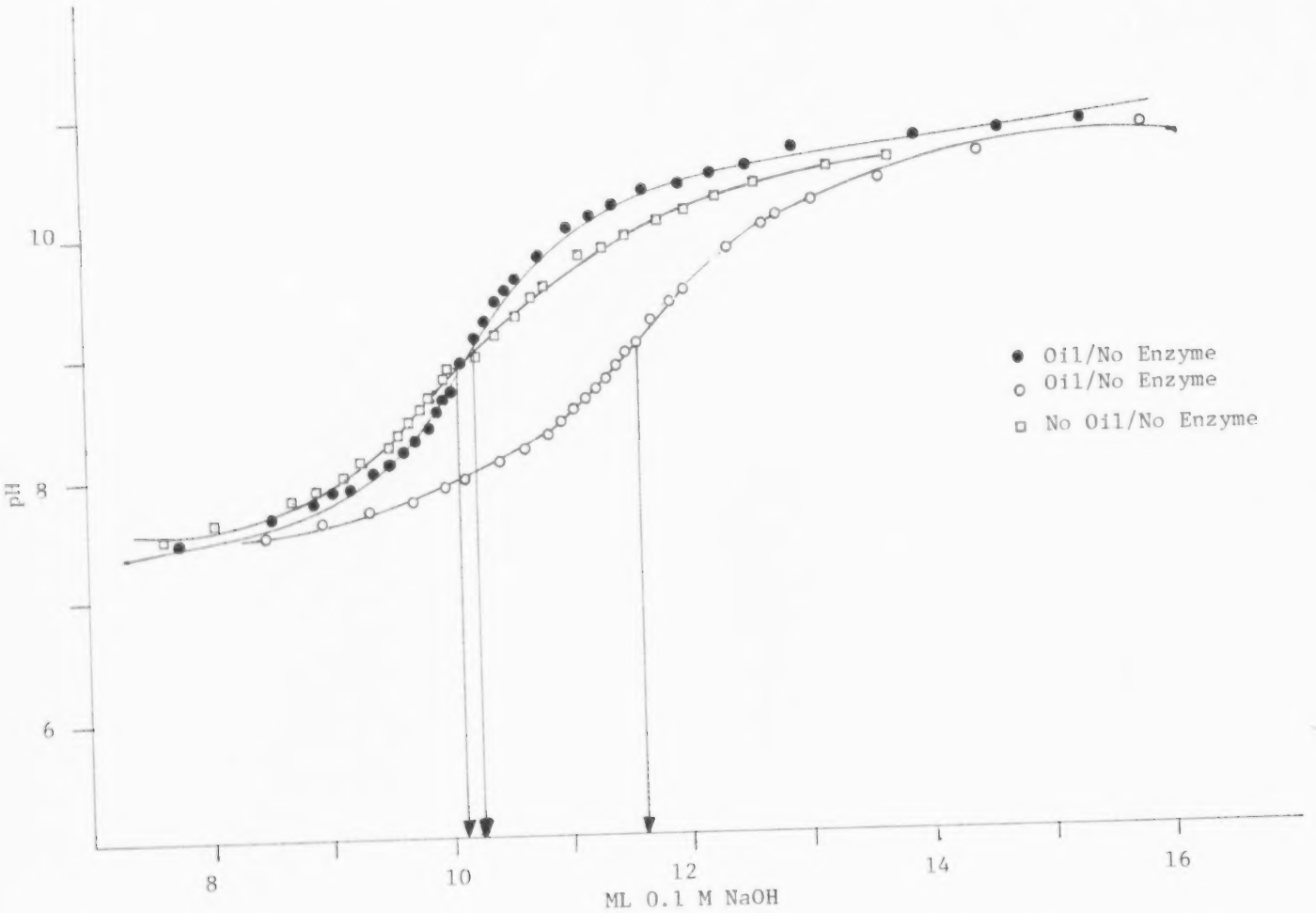


Figure 27. Titration of fatty acids from soybean oil following 2-hr incubation at 40°C.

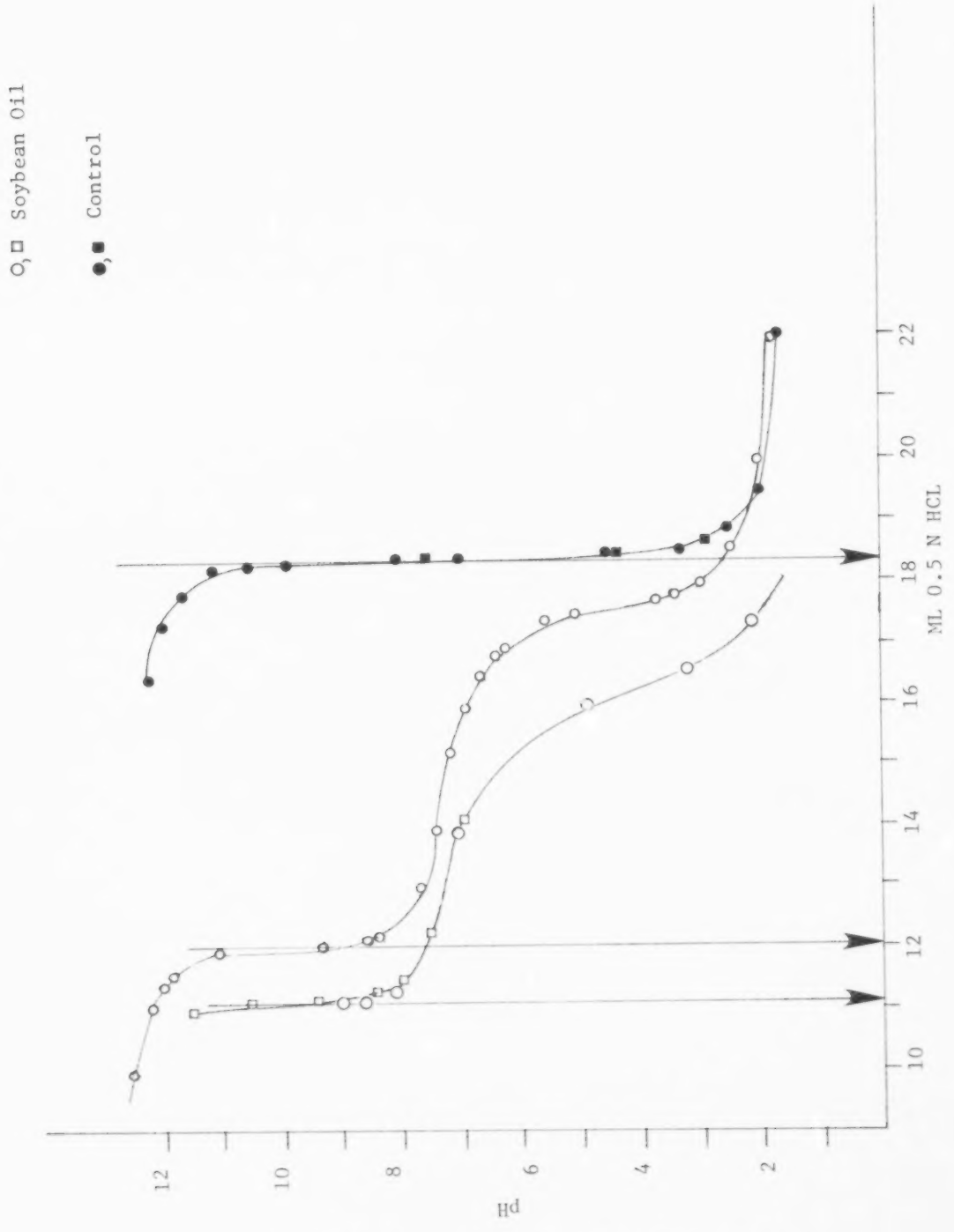


Figure 28. Saponification of soybean oil.

extent of hydrolysis. These data indicated that the enzyme activity, pH, and temperature optima were in the range suitable for direct application to anaerobic sludge. The next task was to apply the enzyme to oil and grease from an anaerobic digester system instead of an artificial, pure substrate.

ENZYME CHARACTERISTICS WITH DIGESTER SKIMMINGS

The next group of experiments examining the effects of lipase enzyme addition utilized skimmings from the primary sludge tank at the Glady Run facility as substrate. This material was chosen because of its high oil and grease content and because it is representative of the natural types of oils and greases to be expected in the anaerobic digester. Analyses of several samples of skimmings indicated that the oil and grease content ranged from 80 to 90 percent of the filtered wet solids. In order to further purify the skimmings sample, it was processed by Soxhlet extraction according to Standard Methods to recover a purified freon soluble oil and grease sample (9). All experiments with skimmings utilized this extract as substrate, and all hydrolysis results were referenced to the saponification of this material as shown in Figure 29. The calculated saponification value for this extract was 128-mg KOH/g extract.

The experimental design for this work consisted of four separate experiments to identify the optimum temperature and pH for hydrolysis as well as the effects of enzyme concentration and incubation time on extent of hydrolysis. In these experiments, each set of test conditions was evaluated in triplicate. In the first experiment, three series of flasks were loaded with enzyme at 1667, 3750, 6667, 10,416, and 41,660 ppm (enzyme/oil wt basis) and incubated at 30°, 35°, and 40°C, under the conditions specified previously in Table 28. Figure 30 shows the linear regression lines computed from the test data. Although 40°C produced the greatest extent of hydrolysis, it was decided to conduct all further experiments at 35°C, the optimum for anaerobic digestion systems.

The second experiment examined the effect of incubation pH on the extent of hydrolysis. These data were obtained at an enzyme loading of 4840 ppm (enzyme/oil wt basis) to provide an easily measured level of hydrolysis following 24 hours incubation at 35°C. Other conditions were as specified in Table 28. Figure 31 illustrates the results obtained in this experiment. The greatest extent of hydrolysis was observed in the pH range 7 to 8. Since the anaerobic digester optimum is approximately pH 7.0, it was decided to conduct all further experimentation at this pH value.

The third experiment in this series examined the effect of enzyme concentration on hydrolysis at 24 hours, pH 7, and 35°C. Other conditions were as specified in Table 29. Enzyme loading levels employed were 658, 3288, 4633, 6577, 18,533, and 37,067 ppm (enzyme/oil wt basis). Figure 32 shows the results of this experiment. The data are plotted here using the empirical relationship described previously in this report for cellulose hydrolysis. In this case an excellent relationship can be drawn up to about 75 percent hydrolysis of the extracted skimmings oil and grease.

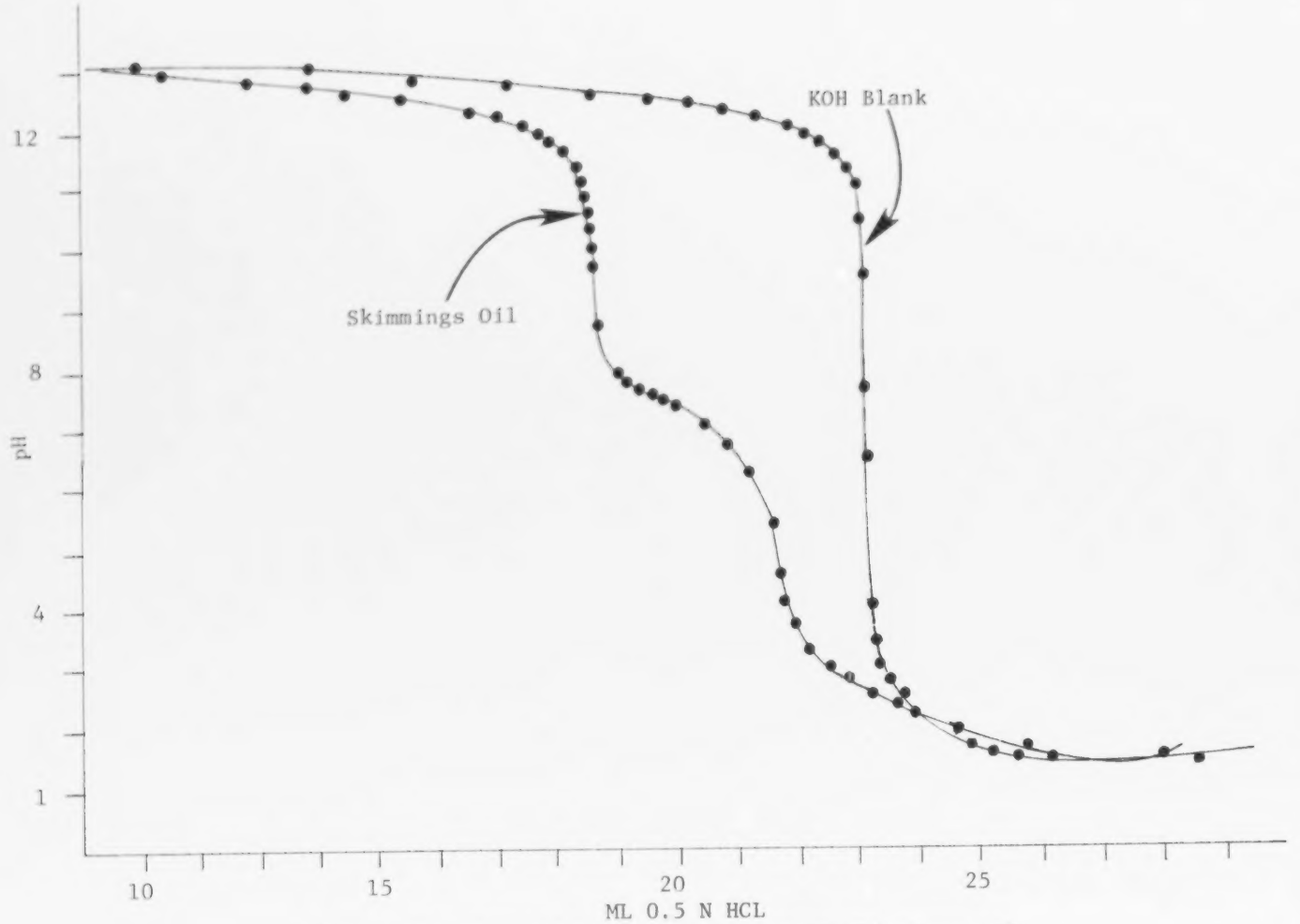


Figure 29. Saponification of extracted skimmings oil.

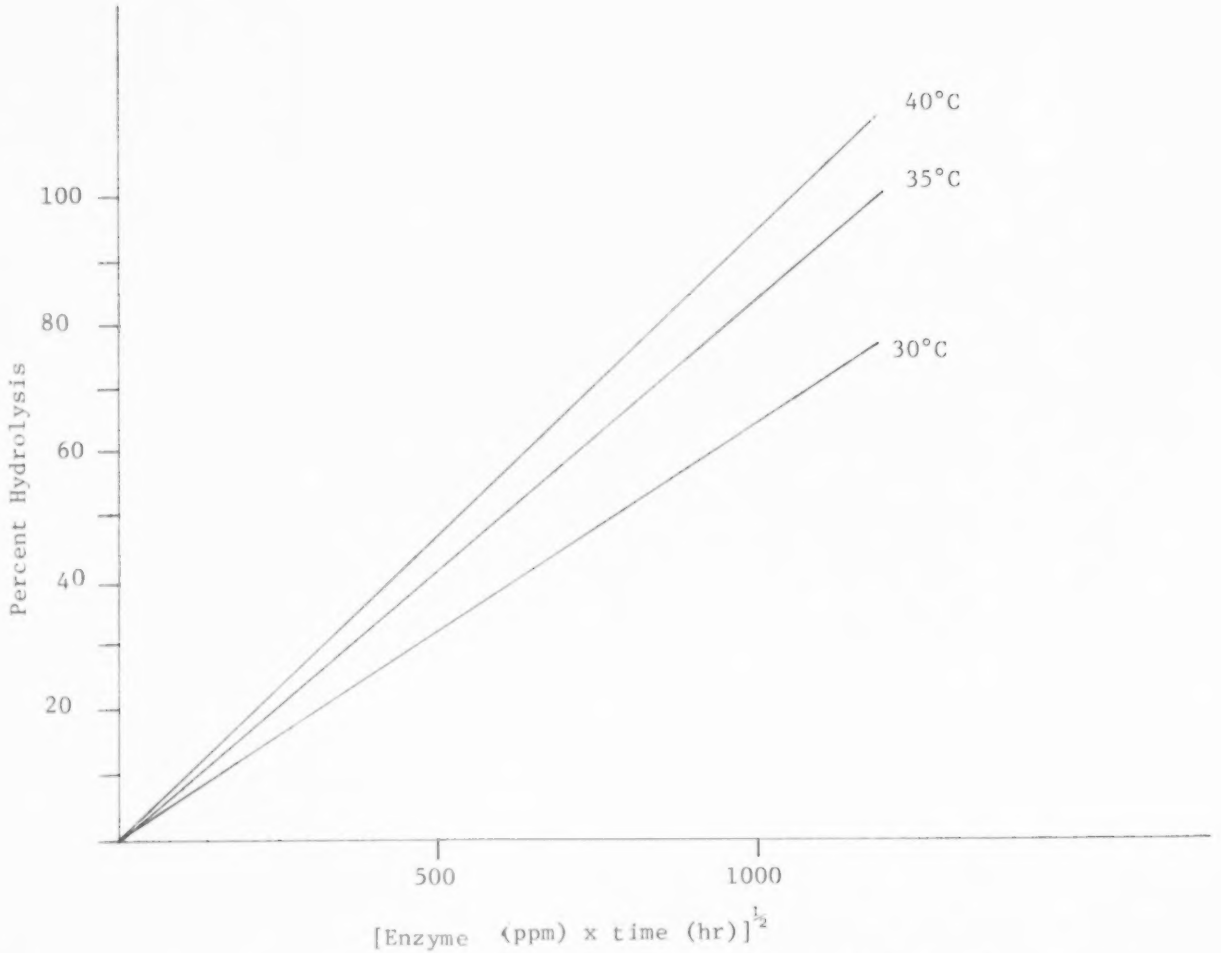


Figure 30. Composite temperature effect on fat hydrolysis of extracted skimmings oil.

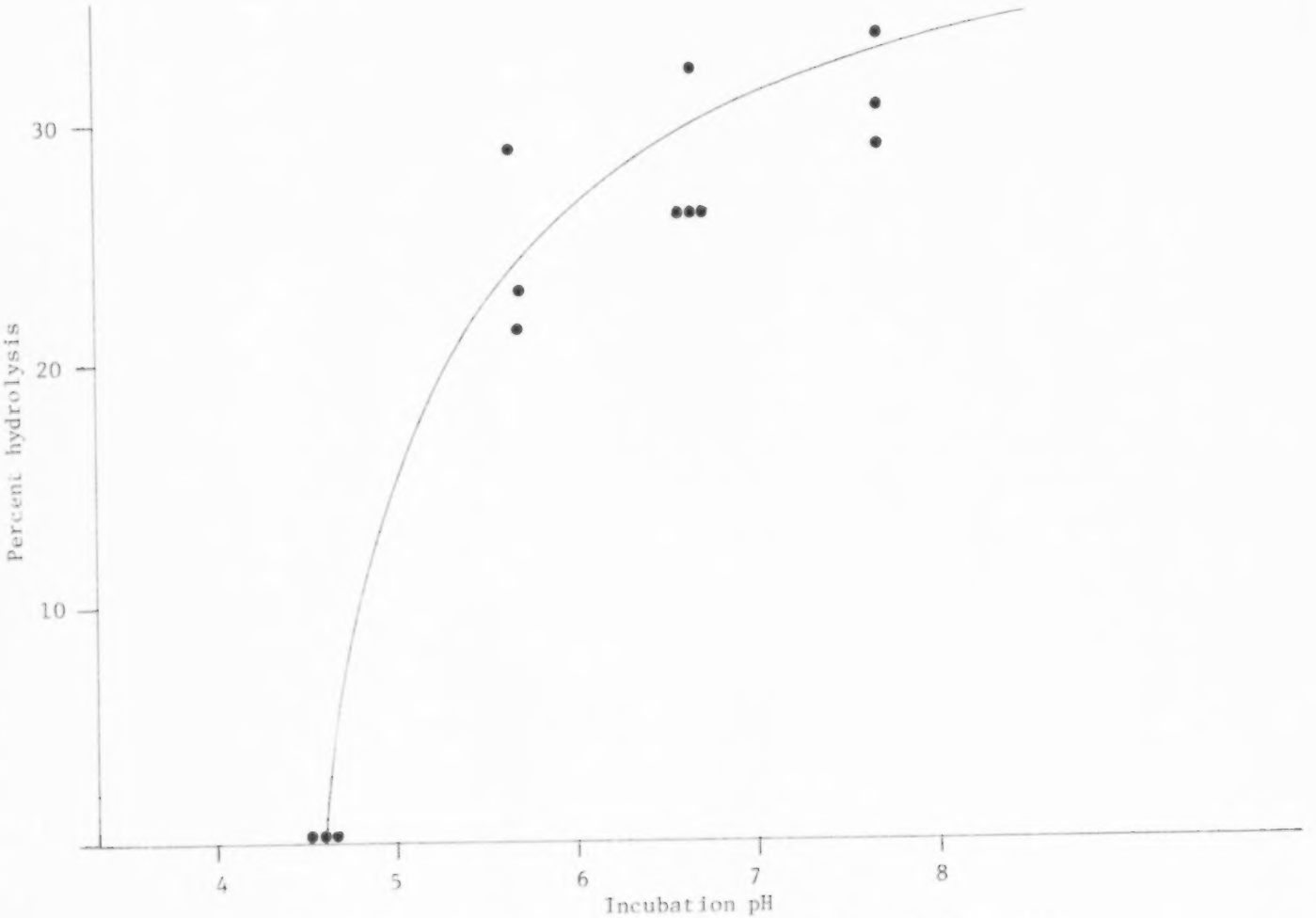


Figure 31. Effect of pH on hydrolysis of extracted skimmings oil.

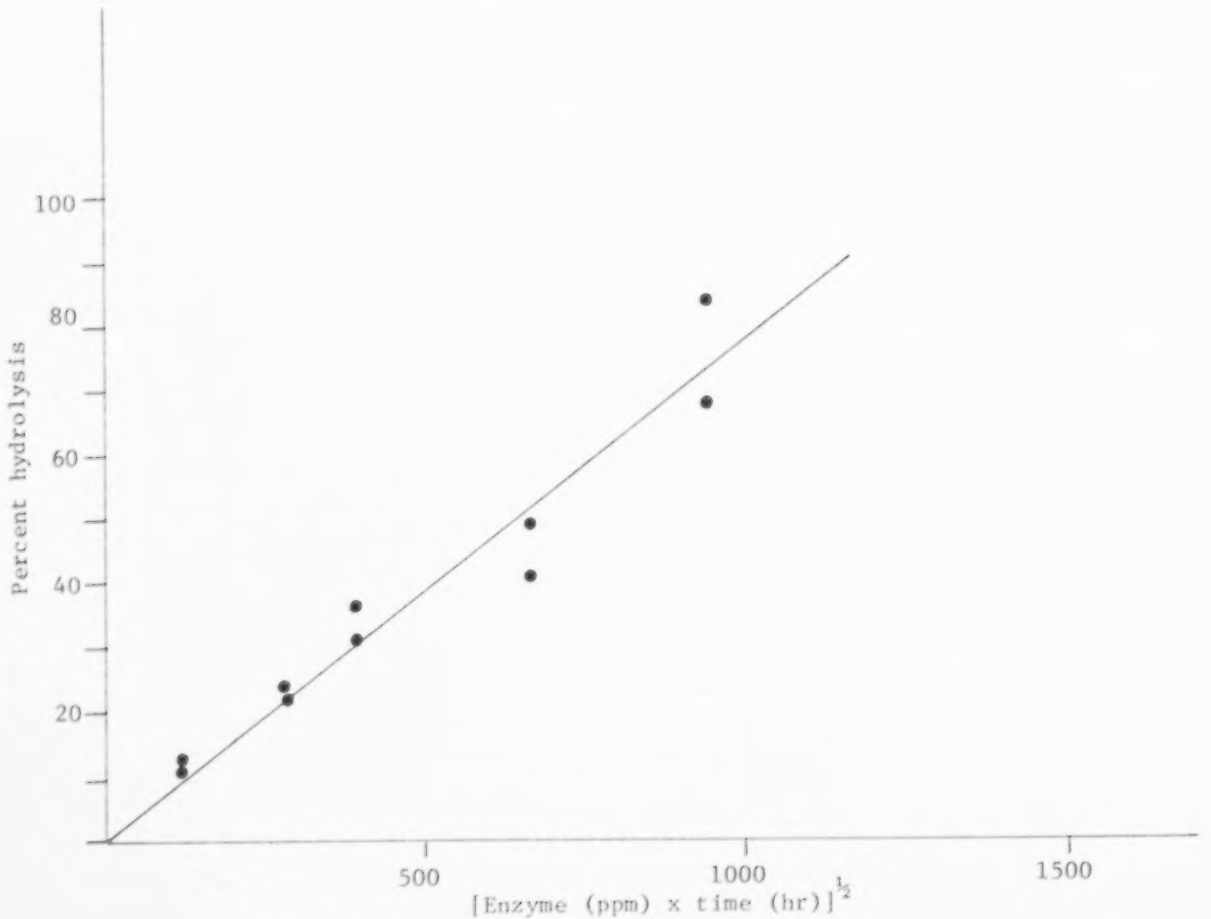


Figure 32. Enzyme concentration effects on oil/grease hydrolysis.

The final experiment in this series examined the effect of incubation time on extent of hydrolysis. This experiment was conducted at pH 7.0, 35°C, and an enzyme loading of 4633 ppm (enzyme/oil wt basis) chosen to provide an easily measured level of hydrolysis. Other experimental conditions were as noted in Table 28. Figure 33 shows the results of this experiment plotted as in Figure 6-6. A clear effect of incubation time on extent of hydrolysis can be observed.

Figure 34 shows a composite of the time and concentration data obtained in the two previously described experiments. The figure illustrates the usefulness of the empirical relationship drawn between extent of hydrolysis and incubation time/enzyme loading. It also provides a predictive tool for estimating the extent of hydrolysis expected as a function of time and concentration, yielding a linear regression equation:

$$\text{Percent hydrolysis} = .0835\ (\text{time} \times \text{concentration})^{1/2},\ r = 0.93$$

OPTIMIZATION STUDY WITH DIGESTED SLUDGE

The final study prior to evaluating lipase enzymes on a process scale examined the batch-scale use of lipase enzymes with digested sludge as substrate. This study differed from the previously described lipase work in that no measures were employed to emulsify the oils and greases or to control the pH beyond the normal buffering capacity of anaerobic sludge. In addition, no external additions of calcium, a suspected activator of the lipase enzyme, were made.

A series of flasks (triplicates at each test condition) containing 100 mL of primary sludge (TS = 1.94%, VS = 78.5%) were spiked with 20 mL of anaerobic digester supernate and loaded with enzyme at 6443 ppm (enzyme/total solids wt basis). They were then nitrogen purged to remove dissolved gases and incubated at 35°C in a Lab-Line® Orbit-Environ Shaker at 3.4 Hz with a 2-cm displacement for intervals of 5, 10, and 15 days. The test effects measured included percent of initial oil and grease remaining and percent replacement of the purge gas with methane.

Figure 35 shows the results of the oil and grease analyses performed on these samples by the Standard Methods Soxhlet extraction method (9). The results of enzyme addition showed a clear increase in hydrolysis throughout the 15-day test period. The most noticeable impact appeared to be on the rate of hydrolysis, however, since at Day 10 the amount of oil and grease remaining in the lipase enzyme-augmented flasks was less than half of that in the control without enzyme. Figure 36 shows that these data were paralleled by the percent replacement of the initial purge gas by methane. At Day 10, the enzyme flasks had approximately 30 percent more methane in the head space gases than did the comparable controls.

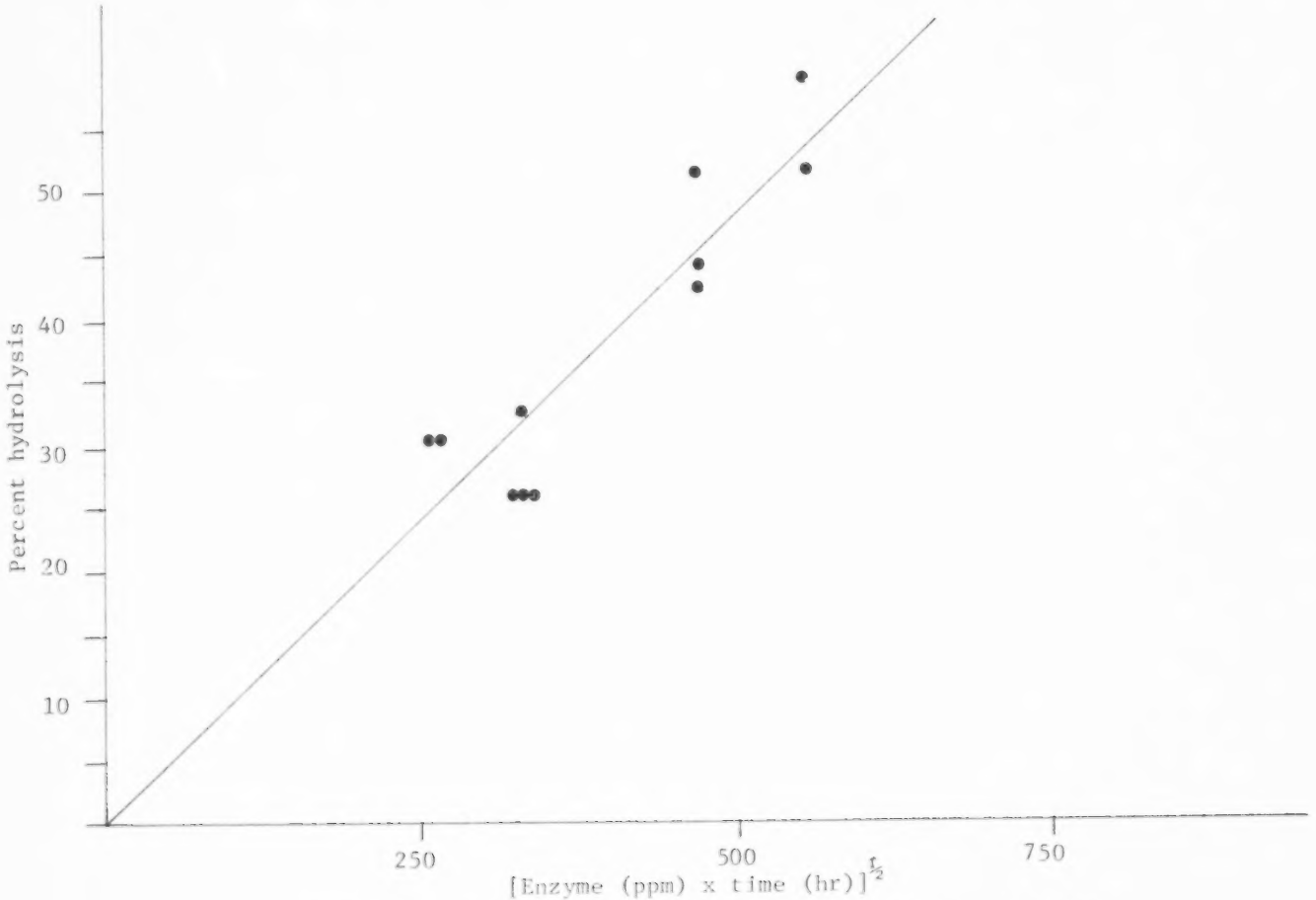


Figure 33. Incubation time effects on oil/grease hydrolysis.

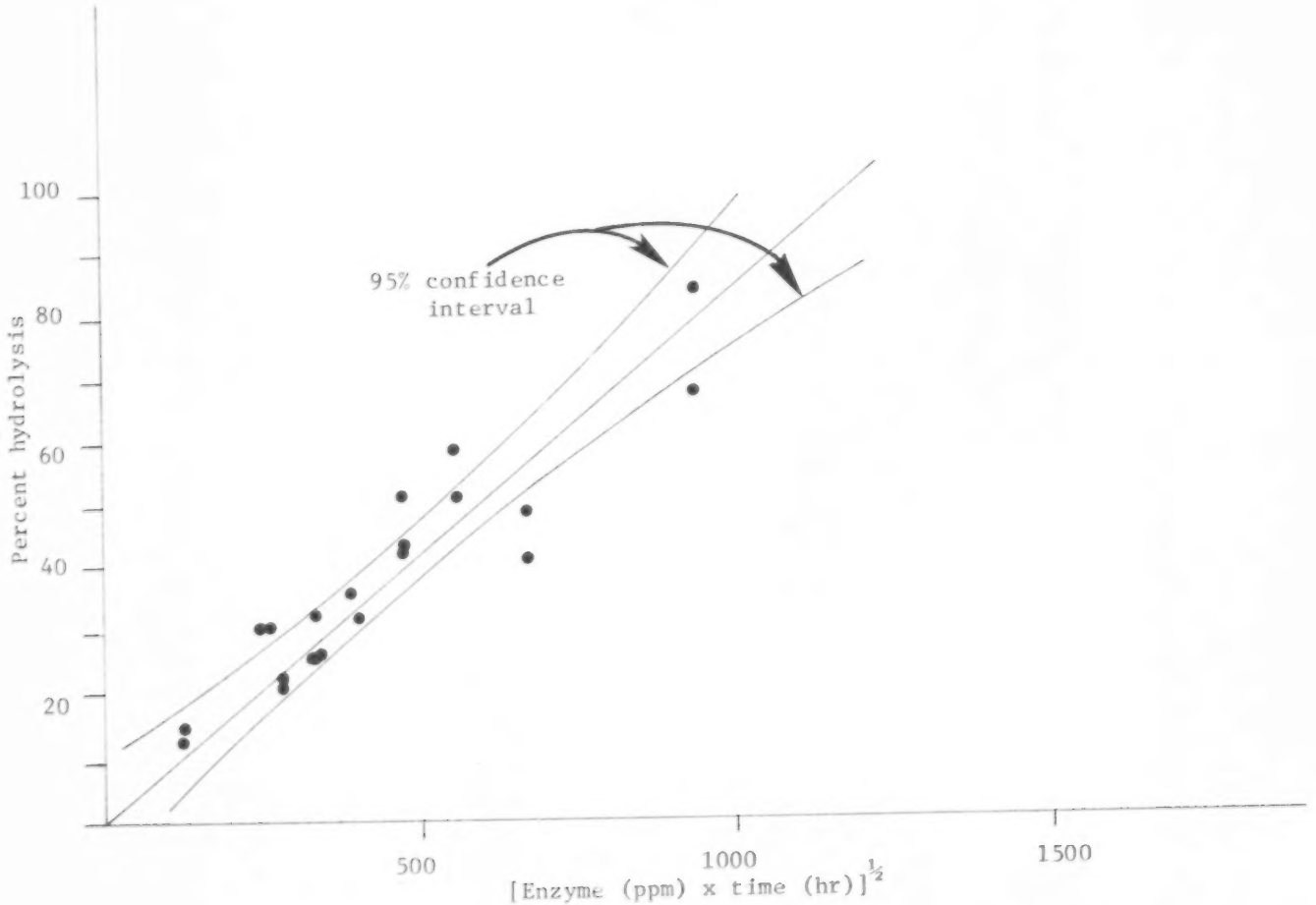


Figure 34. Composite time/concentration effects on oil/grease hydrolysis.

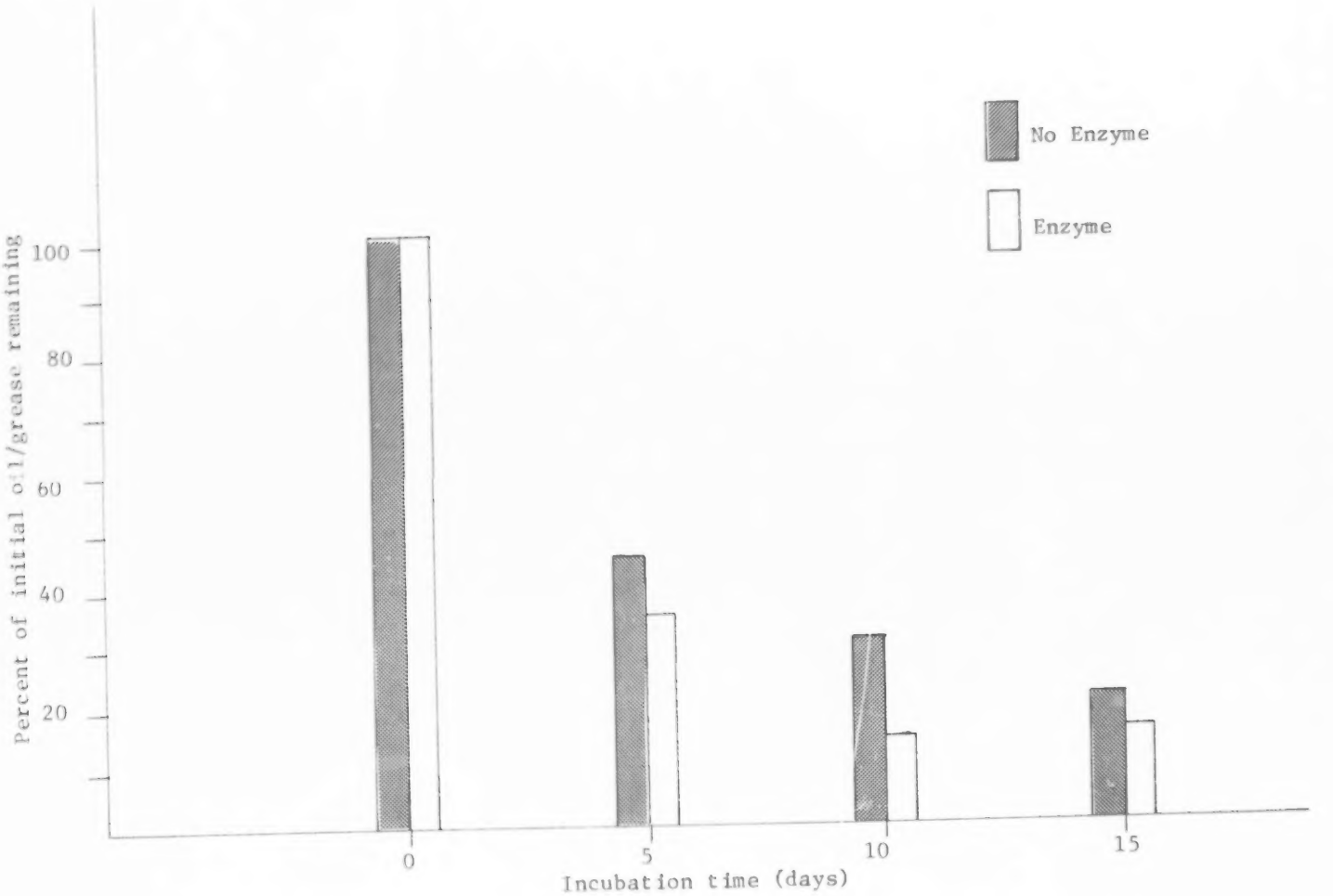


Figure 35. Hydrolysis of oil and grease in anaerobically digesting sludge.

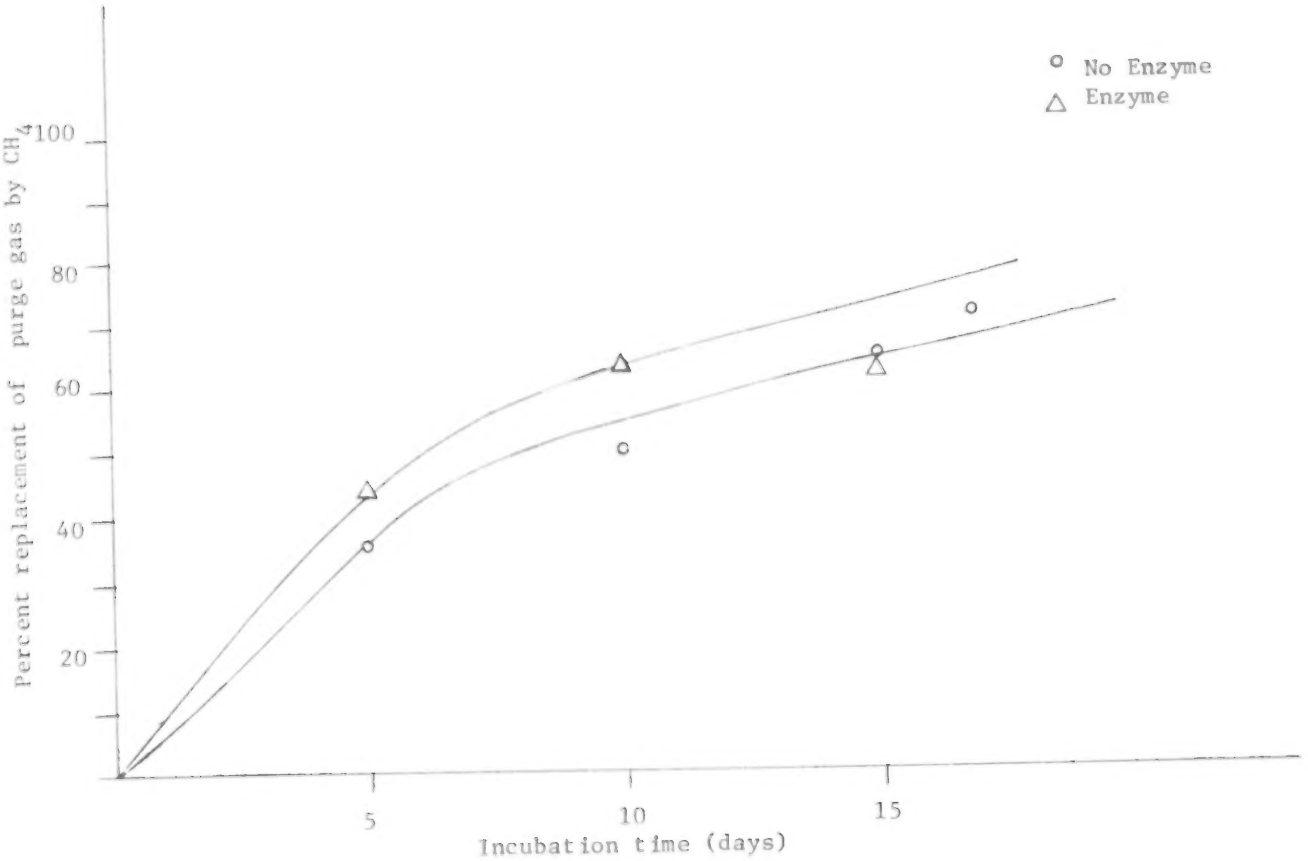


Figure 36. Methane production in anaerobically digesting sludge as a function of enzyme addition.

PROCESS STUDY

The results of the preliminary lipase studies indicated that the temperature and pH of the anaerobic digester would be suitable for simultaneous enzyme treatment and anaerobic digestion. In order to evaluate the process concept over an extended time interval, a laboratory-scale process line study was conducted. Figure 37 provides a schematic illustration of the two laboratory systems employed for this evaluation. The control system differed from the test system in that inactive, heat-denatured enzyme was added to the control, while the test system received active enzyme. The digesters, shown previously in Figure 18, were 6-L glass flasks equipped with gas-tight stoppers connected to gas collection devices by Tygon tubing.

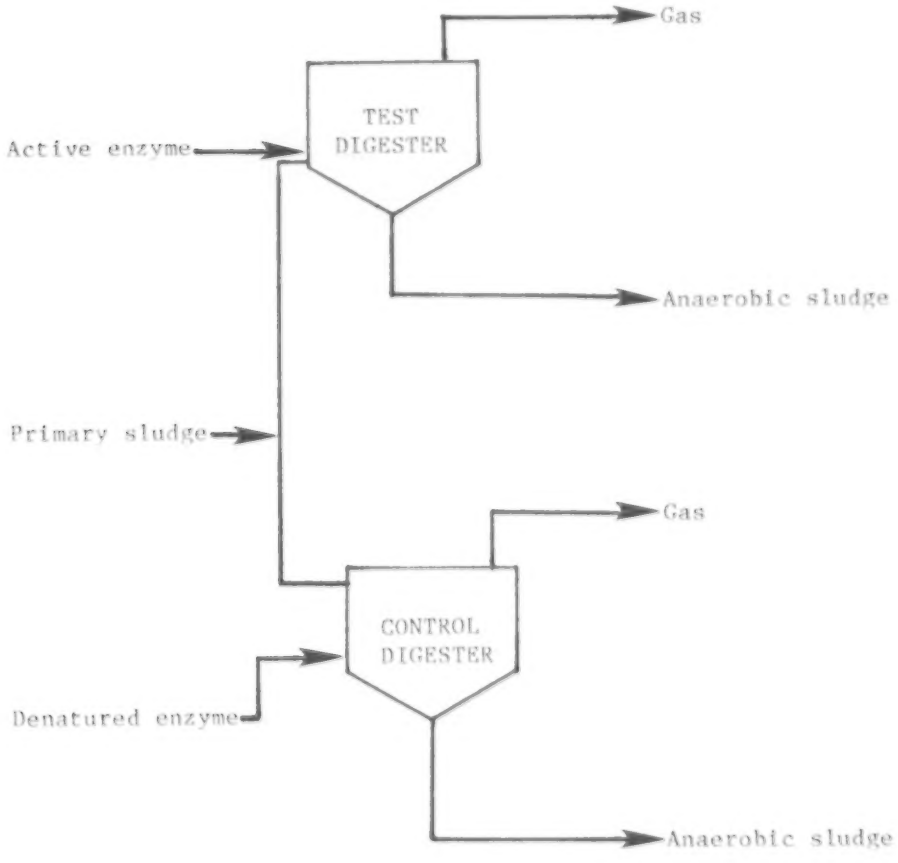


Figure 37. Control and test systems for Lipase Simultaneous Treatment Process Study.

The digesters were maintained in a circulating water bath at 35°C and operated at a 15-day hydraulic retention time. The gas collection spirometers were constructed of PVC pipe and filled with an acidified, salt-saturated gas collection solution. The spirometers were calibrated to permit standard gas volume measurements (60°F and 30 in. Hg).

The test systems were operated as semi-continous flow processes. The test duration was 35 days. Table 29 summarizes the daily volume flows through the systems. A consistent method of operating the test and control systems was established in order to provide as much reproductivity as possible in this experiment. To control the input solids to the reactors, the primary sludge was filtered through a 9.5-mm sieve and adjusted daily to approximately 2.5 percent total solids by dilution with tap water. This value is within the normal range cited for primary sludge (2-7 percent) and yet dilute enough to permit easy handling. (4). Characteristics of the feed sludge are shown in Table 30. The digesters were fed and gas production recorded once each day at approximately 1200 hours. The digesters were thoroughly mixed by shaking three times each day at approximately 0800, 1200, and 1700 hours.

TABLE 29. DAILY VOLUME FLOWS FOR LIPASE SIMULTANEOUS TREATMENT PROCESS CONCEPT

Steam	Control system (mL)	Test system (mL)
Primary sludge	300	300
Enzyme	5*	5
Digester influent†	300	300
Digester effluent	300	300

* Heat-denatured enzyme.
† Digester was maintained at 4500 mL with a 15-day hydraulic retention time.

As was done in the cellulase study, the experimental design employed to conduct the process study was formulated to test the following hypothesis: is the performance of the test system better than that of the control? Several measures were employed to permit a valid assessment of this hypothesis. The test and control systems were operated identically before and after the test phase with no enzyme addition for at least two hydraulic retention times (30 days). It was required that solids destruction and gas production in the systems not be significantly different ($\alpha = .05$) during this period. All data taken during the testing phase were analyzed on a daily or weekly basis utilizing a t-test for paired observation with an $\alpha = .05$ (7). This approach was taken to minimize the effects of the day-to-day variability in primary sludge composition and subsequent systems performance. During this

study, enzyme was added to the systems at a mean concentration of 4950 ppm by weight (0.495 percent by weight) based on the results of the optimization studies.

TABLE 30. PRIMARY SLUDGE CHARACTERISTICS FOR LIPASE SIMULTANEOUS LINE STUDY PROCESS

Characteristic	Mean value
TS (percent)*	2.44
TVS (percent of TS)	80.54
pH	5.8
Heat content (Btu/lb)	7490

* Adjusted by dilution with tap water.

Table 31 summarizes the analyses performed to evaluate the test hypothesis for the lipase process study. The various analyses listed were performed to permit evaluation of solids destruction and gas production, assessment of general system health, and calculation of mass and energy balances for scale-up to a commercial-scale process. The latter analysis is described in more detail in Section 7 of this report.

Table 32 summarizes the mean values of the solids and gas production data obtained during the lipase process study. All test data are shown in the Appendix. Also included in this table is a statement regarding the presence of statistically significant ($\alpha = .05$) differences between the control and the test systems' data. This information was determined by computing daily differences between the systems and applying paired t-test (7).

No significant differences ($\alpha = .05$) were observed between the test system and the control system for total and volatile solids either entering or leaving the digesters. Significant differences ($\alpha = .05$) were observed in the gas production resulting from the test digester. The data in Table 32 show increases over the control of 3 and 5 percent for total gas and methane production per unit weight volatile solids input. It is suspected that greater increases in gas production would have been observed had the digester retention time been extended. Data reported in the literature have shown that very little destruction of fatty acids occurs until at least 10 days detention time (25).

Table 33 presents mass and energy balance calculations for the solids and gas production data shown in Table 32. The mass balances are quite good for both test and control systems. The energy balance calculations are

TABLE 31. ANALYSIS SCHEDULE FOR LIPASE SIMULTANEOUS TREATMENT

	TEST SYSTEM					CONTROL SYSTEM			
Analysis	Xenia primary sludge	Digester in	Digester out	Filter cake	Filtrate	Digester in	Digester out	Filter cake	Filtrate
TS	D		D	C			MWF	C	
VS	D		D	C			MWF	C	
pH	D		D				D		
Gas volume			D				D		
CH_4 percent			D				D		
CO_2 percent			D				D		
Volume	D	D	D		C	D	D		C
Weight	D	D	D	C		D	D	C	
Enzyme weight									
Volatile acids			MWF				C		
Alkalinity			MWF				C		
TKN			C				C		
Total P			C				C		
BOD			CX				CX		
Btu/lb	C		C	C			C	C	
Filter yield			C				C		
Specific resistence			C				C		
Cellulose	C								

D = daily
MWF = Monday, Wednesday, Friday
C = Weekly composite
CX = Weekly composite, analysis performed by Xenia

TABLE 32. SOLIDS AND GAS PRODUCTION DATA FOR THE LIPASE PROCESS STUDY (Daily Basis)

Process stream	Control system	Test system	Significantly different (α = .05)
Primary sludge			
TS (g)	7.32	7.32	No
TVS (g)	5.90	5.90	No
Enzyme			
TS (g)	0.0218*	0.0218	No
Anaerobic digester effluent			
TS (g)	4.41	4.42	No
TVS (g)	2.99	3.02	No
Anaerobic digester gas production			
Gas L/g TVS input†	.527	.543	Yes
L§	3.11	3.20	Yes
g**	3.34	3.39	Yes
CH_4 L/g TVS	.351	3.68	Yes
L§	2.07	2.17	Yes
g**	1.40	1.47	Yes
CO_2 L§	1.04	1.03	No
g**	1.93	1.92	No

* Heat denatured enzyme.
† Liters per gram TVS input.
§ Measured volume.
** Calculated from composition.

somewhat less satisfactory than the mass balances. It is felt that the input energy values may be somewhat low since heating values were computed for oven-dried sludge. Oven-dried sludge has been observed to be artificially low in certain fractions of the volatile solids such as oils and greases (9).

Figures 38 and 39 summarize the results of analyses for pH, volatile acids, and alkalinity in the test and the control digesters. All values were within the normal ranges reported for digested sludge: pH 6.5 to 7.5, volatile acids 100 to 600 mg/L as HOAc, and alkalinity 2500 to 3500 mg/L as $CaCO_3$ (4). However, volatile acids were relatively low for normal digestion systems.

TABLE 33. MASS AND ENERGY BALANCES FOR LIPASE SIMULTANEOUS TREATMENT LINE

Process stream	Control system		Test system	
	Mass (g)	Energy (Btu)	Mass (g)	Energy (Btu)
Input				
Primary sludge	7.32	121	7.32	121
Enzyme	.02	---	.02	---
Total	7.34	121	7.34	121
Output				
Biogas*	3.34	73	3.39	77
Digested sludge	4.41	71	4.41	71
Total	7.75	144	7.80	148
Balance $\frac{\Sigma output}{\Sigma input} \times 100$	106%	119%	106%	122%

* Heat content of methane assumed at 1000 Btu/ft^3.

Table 34 shows the results of analyses for total Kjeldahl nitrogen and total PO_4 - P in digested sludge for the test and control systems. There were no significant differences (α = .05) between test and control systems for these data. The TKN values represent a total solids nitrogen content of about 1 percent. This is somewhat below the normal range for digested sludge, 1.6 to 6 percent of total solids (4). Both digesters also showed ratios of N/P on the order of 10 to 15:1. This ratio is slightly in excess of the normal ratio expected for anaerobic metabolism, which is on the order of 5:1 (35,36).

Table 35 summarizes the results of additional analyses conducted on the digester effluents during the Lipase Simultaneous Treatment Process Study. Shown in this table are the average results of BOD analyses, filter yield, and specific resistance measurements, as well as heat content analyses of the filter cakes from the filter yield studies. The filtration studies were performed as described by Vesilind (37). The results of the BOD analyses

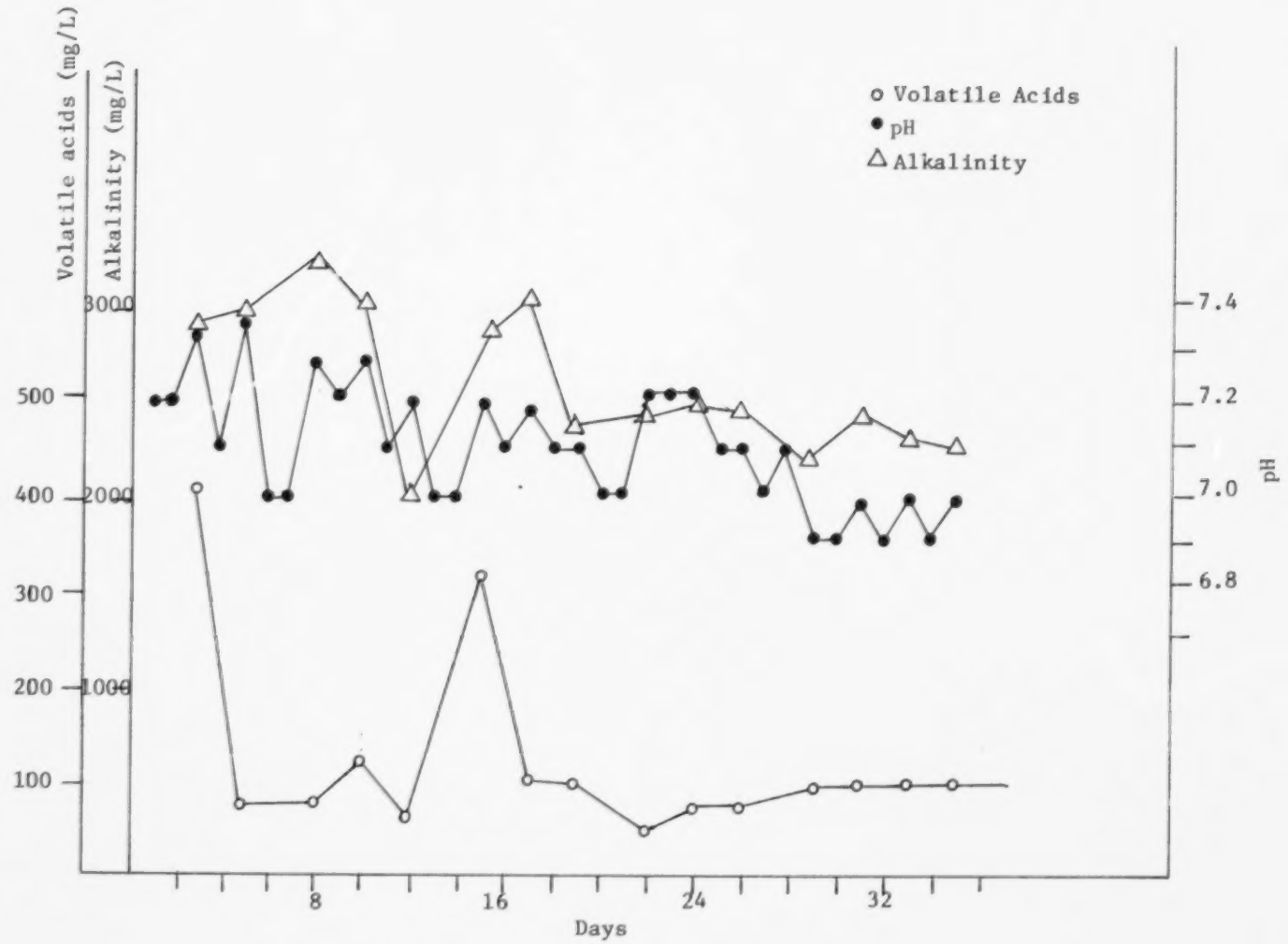


Figure 38. Lipase test system digester data.

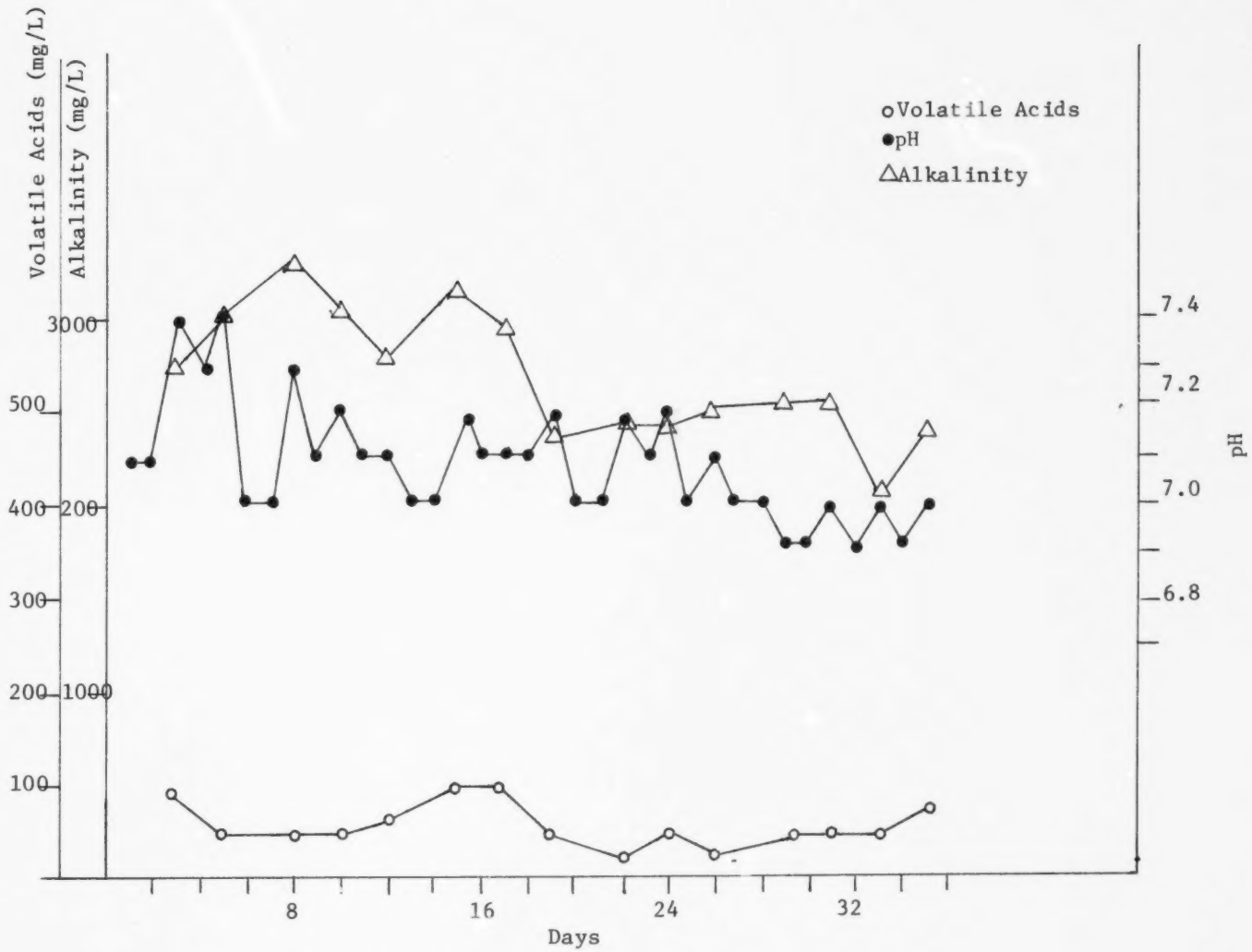


Figure 39. Lipase control system digester data.

TABLE 34. ANALYSIS OF TEST AND CONTROL LIPASE SIMULTANEOUS TREATMENT DIGESTERS FOR TKN AND TOTAL P

Parameter	Control (mg/L)	Test
TKN (NH_3 as N)	268	300
Total P (PO_4 as P)	19	26

TABLE 35. BOD, FILTRATION STUDIES, AND HEATING VALUE ANALYSIS FOR THE LIPASE SIMULTANEOUS TREATMENT PROCESS STUDIES

Characteristic	Control system	Test system	Significantly different (α =.05)
Digested sludge (BOD [mg/L])	1961	1981	No
Filter yield (lb/ft^2/hr)	7.9	8.5	No
Specific resistance (m/kg)	29.6	32.4	No
Filter cake heating value (Btu/lb dry)	7353	7445	No

showed that the test and control digesters were not significantly different (α = .05). The results of the filtration studies are of use primarily as a means to compare the sludge characteristics of the test and control systems. The data cannot be used directly for design purposes since appropriate filter media were not available for these tests. Nevertheless, the data demonstrate that no significant differences (α = .05) were observed in filter yield and specific resistance measurement between the test and control digesters. In addition, enzyme hydrolysis was found to have no significant effect (α = .05) on the heating value of the filter cakes derived from the filter yield studies.

Table 36 summarizes the salient performance characteristics of the control and test systems during the lipase study. These data indicate that only a minimal enhancement of the digestion process results from lipase enzyme addition.

TABLE 36. PERFORMANCE CHARACTERISTICS OF LIPASE PROCESS SYSTEMS

	Control system	Test system
Temperature (°C)	35	35
Solids loading (g VS/L)	1.3	1.3
Detention time (days)	15	15
Feed TS (%)	2.44	2.44
Feed VS (%)	80.6	80.6
Biogas production		
Rate (vol/vol/day)	.69	.71
Yield (standard m^3/kg VS added)	.53	.54
(standard m^3/kg VS destroyed)	1.08	1.10
CH_4 production		
CH_4 (%)	67	68
Rate (vol/vol/day)	.46	.48
Yield (standard m^3/kg VS added)	.35	.37
(standard m^3/kg VS destroyed)	.71	.76
Efficiencies		
VS reduction (%)	49	49
Feed energy recovery as CH_4 (%)	60	64

SECTION 6

TECHNICAL AND ECONOMIC EVALUATION OF PROCESS CONCEPTS

METHOD OF COMPARISON

The final objective of this program was to assess the impact of the research results on current operations at Xenia's Glady Run facility. This assessment was accomplished by first scaling the results of the cellulase and lipase programs to a 2-million gallon per day (MGD) facility equivalent to the Glady Run plant. The technical program data were then used to determine whether process modifications or additional equipment would be required. Finally, an economic assessment of the process concepts was performed as a comparison to operations at the City of Xenia facility. The technical data obtained in this program suggested that the cellulase pretreatment process concept and the lipase simultaneous process concepts were most suitable for consideration in this assessment. These two concepts as well as current operations at the Glady Run facility therefore form the basis of the technical and economic evaluation.

In order to provide a useful technical and economic assessment of the impact of enzymatic hydrolysis, three sets of data are presented for the cellulase process concept. The primary standard of comparison is the baseline for the Xenia Glady Run facility as described in the following paragraphs. Scaled-up data from the control and test systems used in the process studies are also included. The control system's data represent changes in performance due to deviations in operating conditions from normal Xenia baseline. The test system's data represent changes in performance due to differing operating conditions as well as the addition of active enzyme to the systems. Each of these data sets is described in more detail in the following comparisons.

XENIA GLADY RUN BASELINE CHARACTERISTICS

The Xenia Glady Run facility represents a significant part of Xenia's wastewater treatment capabilities, treating approximately 2 MGD of municipal wastewater. Figure 40 provides a schematic representation of the facility. Although several options exist for plant operations, the facility employs activated sludge treatment with anaerobic digestion of the primary clarifier solids and aerobic digestion of the waste activated sludge.

Wastewater entering the plant is first screened in a grit chamber prior to entering the primary clarifiers. Residence time in the primary tanks is approximately 2 to 3 hours. Solids collected in the primary tanks are pumped at least twice daily to the first anaerobic digester. This vessel is heated

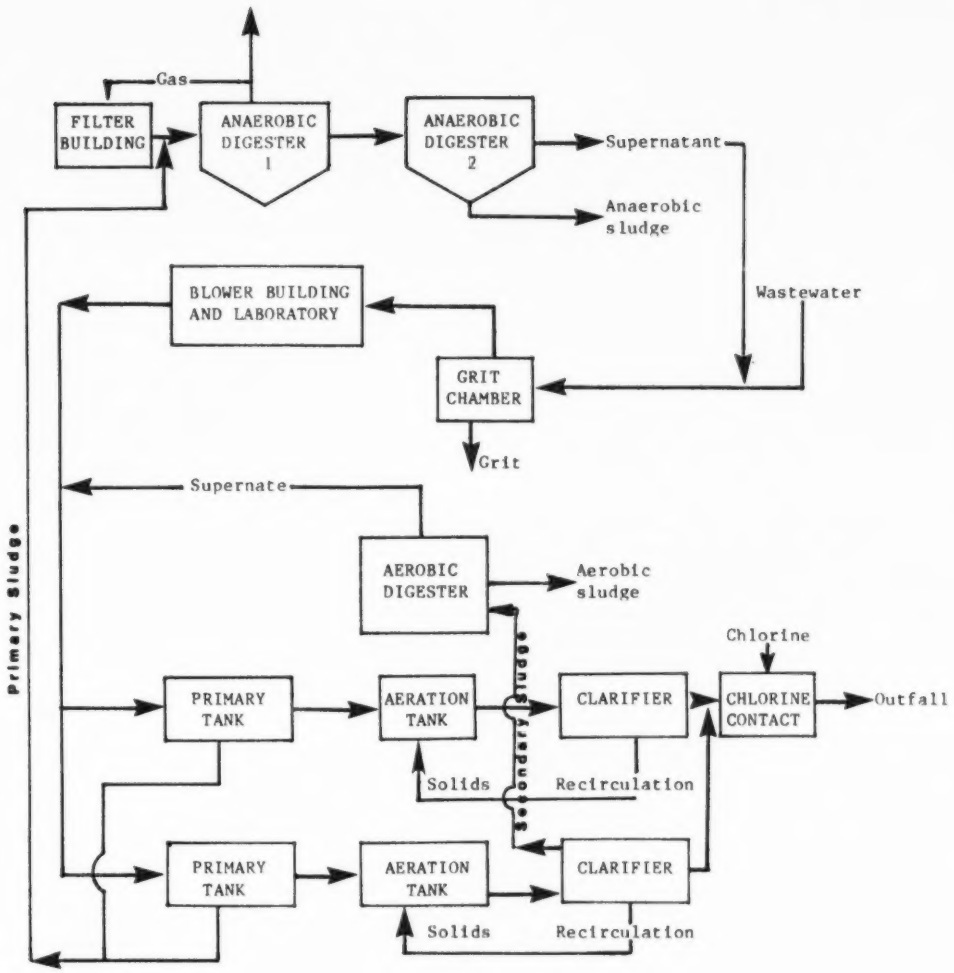


Figure 40. Xenia Glady Run Facility.

to approximately 35°C (95°F) by sludge circulation through a boiler/heat exchanger system operated on methane gas generated in the digester. The two digesters (250,000 gallons each) are operated in series, and together can provide a variable residence time approaching 70 to 80 days. The second digester is not heated and has not been noted to produce biogas. This digester functions essentially as a holding tank prior to solids removal for land disposal. Supernatant is removed periodically from the second digester and reintroduced at the head of the plant.

The effluent from the primary tanks flows to the activated sludge system, which operates at a hydraulic retention time of approximately 10 hours. The effluent is then transferred to the final clarifiers where solids are removed for further treatment. The effluent is subjected to a final chlorine contact process prior to exiting at the plant outfall to Glady Run. Solids from the final clarifiers are transferred to an aerobic treatment system prior to ultimate land disposal. The supernate from this system is periodically reintroduced to the primary treatment tanks.

In order to identify mass and volume flows through the total system, it was necessary to supplement the information provided by the City of Xenia with specific reference values and engineering judgment. This was required because Xenia does not collect some of the data relevant to the present research program since it is not crucial to their own normal operations. The analysis utilized data from the City of Xenia on the following parameters: wastewater and outfall volumes, total solids, and BOD; and total solids concentrations in the primary, secondary, and anaerobic sludges. The intermediate mass and volume flows were calculated using the procedures outlined in Vesilind with the assumption that total solids are equivalent to suspended solids (37). Table 37 summarizes the data, assumptions, and reference values utilized. Table 38 summarizes the resultant operating conditions determined for the baseline Glady Run System. Operating conditions are presented in terms of pound solids/pound MWW solids or gallons/gallon MWW, each reference to an input of 2 MGD at 200 mg/L TS and 200 mg/L BOD.

In order to compare the experimental data to this reference point, two further important assumptions were made. First, since the second Glady Run digester functions essentially as a storage tank, it was assumed that in effect only one digester is used which operates consistently at a 15-day retention time. Second, it was assumed that the simplified process flow diagram shown in Figure 41 represents those portions of the Glady Run Facility most relevant to the proposed process concepts. This simplified version of the Xenia facility is hereafter referred to as the Xenia Baseline facility.

CELLULASE PRETREATMENT PROCESS CONCEPT

Technical Evaluation

Figure 42 illustrates the simplified Glady Run schematic in comparison to the cellulase pretreatment process concept. The major difference between

TABLE 37. DATA, ASSUMPTIONS, AND REFERENCE VALUES UTILIZED TO CALCULATE MASS AND VOLUME FLOWS FOR THE GLADY RUN FACILITY

Item	Value
MWW input	
Flow rate	2 MGD
BOD	200 mg/L
TS	200 mg/L
Primary sludge	
Fraction MWW TS removed in clarifier	0.6*
TS	4%
VS	80%
Fraction BOD not removed in clarifier	0.7*
Anaerobic Sludge	
TS	5%
VS	67%
VS destruction	50%†
Biogas CH_4 fraction	70%†
Secondary sludge	
TS	3%
VS	57%
Fraction BOD not removed	0.1%*
Yield	0.5*
Net solids produced from activated sludge process	63 mg/L
Aerobic sludge	
TS	4%
VS	40%
VS destruction	50%†
Outfall	
BOD	20 mg/L
TS	20 mg/L

* Reference values (37).

† Assumptions. All other values obtained from City of Xenia or calculated by SYSTECH.

TABLE 38. ASSUMED GLADY RUN OPERATING CONDITIONS

	lb solids* / lb MWW solids	gallons† / gallon MWW
Inputs		
Municipal wastewater from sewer	1	1
Anaerobic digester supernate	---	.0016
Aerobic digester supernate	---	.0019
Solids synthesized§		
Solids produced in activated sludge process	.32	---
	1.32	1.0035
Outputs		
Outfall	.10	.9999
Anaerobic digester		
Sludge	.36	.0014
Gas	.24	
Aerobic digester		
Sludge	.44	.0022
Gas	.18	---
	1.32	1.0035

* Pound solids in each stream normalized to 1-lb input MWW solids.
† Gallons in each stream normalized to 1-gal input MWW.
§ This item represents conversion of volatile solids to biological solids in the activated sludge process.

these systems involves addition of a pretreatment vessel for enzyme application. Table 39 presents the technical comparison of data obtained from the control and test systems with the Xenia Baseline. The data shown have been directly scaled up from the experimental data of this report, using a mass ratio basis. In order to correct for the fact that slight deficiencies were observed in the mass balances discussed previously, the data in Table 39 have been recalculated, setting the difference between input total solids and measured gas production equal to the output total solids in the digested sludge. All results are reported on the basis of primary sludge input. Shown in this table are only those data relating directly to the City of Xenia'a major objectives in the operation of the facility: production of a minimum amount of sludge suitable for land application and a sufficient amount of methane to heat the digester vessel.

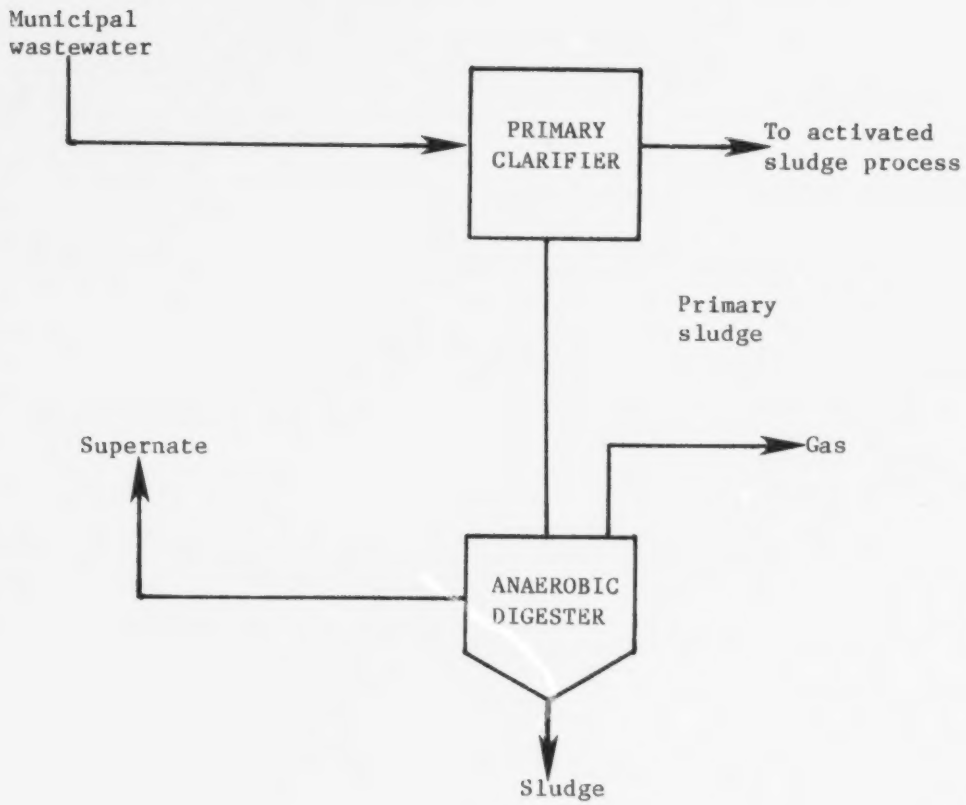


Figure 41. Simplified process flow diagram of Glady Run anaerobic digester system.

It is obvious that adding a pretreatment vessel to the process results in a significant increase in solids reduction and methane production. This result has been observed previously by other researchers in two-stage digestion studies using various substrates (38,41). It is generally felt that this phenomenon results from the development of the pretreatment vessel into an acid-phase digester. The acid-phase digester promotes rapid hydrolysis of organic compounds which can then be converted more efficiently to methane in the second-stage methanogenic digester (38).

The data illustrated in Table 39 indicate that total solids reductions (digested/primary sludge basis) of 40, 58, and 64 percent were observed for the current Glady Run Facility and the hypothetical two-stage (control) and enzyme-augmented two-stage (test) Glady Run facilities. Similar comparison between cubic feet methane production/pound of primary sludge show increases of 144 and 166 percent for the control and test systems over the

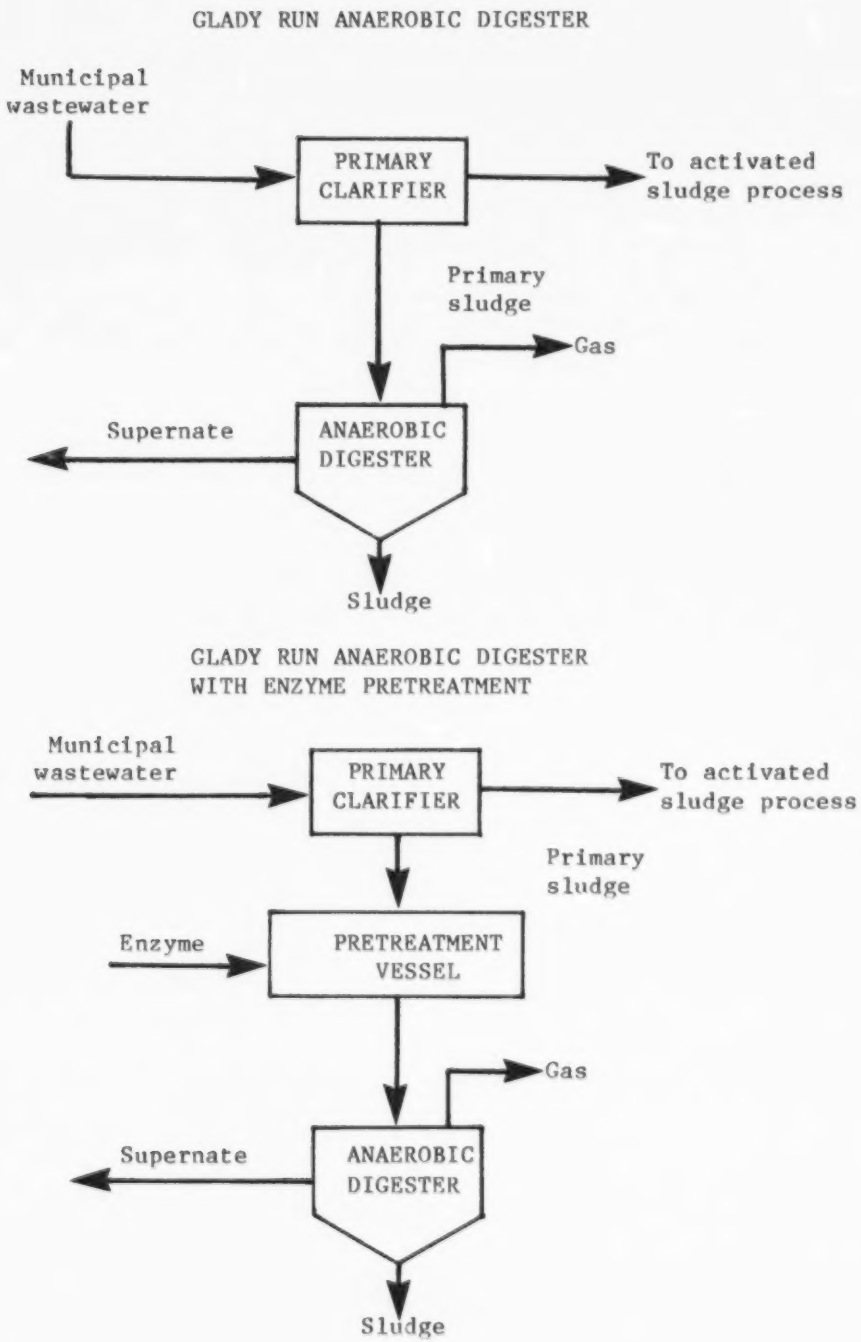


Figure 42. Current and cellulase enzyme augmented Glady Run anaerobic digestion configurations.

current operational facility. Using values of 7490 Btu/lb primary sludge and 1000 Btu/ft^3 methane gas, feed energy recovery efficiencies of 54, 79, and 91 percent can be calculated for the three systems. As expected, the largest improvements are seen to be due to staging rather than enzyme addition.

TABLE 39. XENIA BASELINE AND SCALED-UP CELLULASE PROCESS DATA SUMMARY

Item*	System: Xenia Baseline facility	System: Scaled-up cellulase control system	System: Scaled-up cellulase test system
lb digested sludge total solids/lb primary sludge total solids	.60	.42	.36
ft^3 gas produced/ lb primary sludge	6.04	8.72	9.76
ft^3 CH_4 produced/ lb primary sludge	4.08	5.89	6.78
ft^3 CO_2 produced/ lb primary sludge	1.96	2.83	2.98

Economic Evaluation

The economic analysis of applying a cellulase pretreatment unit operation at the Glady Run facility is based on the simplified process flow diagram and scaled-up operating data shown in Figure 41 and Table 39. This analysis focuses on two key points: solids reduction and methane production. The City of Xenia currently has special local arrangements for the agricultural land disposal of sludge at approximately 5 percent solids. In addition, there is currently no method to utilize methane gas in excess of digester heating requirements.

These two factors complicate the performance of an economic assessment which is relevant both to Xenia and to the technical community at large. It has therefore been assumed for the assessment that the Xenia facility dewaters sludge before disposal at a conventional landfill. It has also been assumed that excess methane can be considered a credit based on its replacement value

for conventional utility energy costs. Typical costs for solids dewatering, solids disposal, and methane credit have been assumed at $20/ton solids, $8/ton solids at 20 percent solids, and $5.5/$10^6$ Btu as methane. The cost for enzyme augmentation has been based on a value of $2.25/lb celluclast supplement with 0.1 lb cellobiase. This projected enzyme cost has been obtained from Novo Laboratories Inc. for commercial grade product in bulk quantity (42). An enzyme loading of 2900 ppm (weight of enzyme/weight of primary sludge TS) for 24 hours at 35°C has been assumed. This loading is based on process data and optimization of the relationship between enzyme loading and incubation time shown previously in Figure 16.

Table 40 illustrates the economic assessment of the simplified Xenia Baseline facility and the scaled-up test and control system. Net savings per million gallon wastewater of $15.25 and $17.34 are shown for the control and test systems, respectively. These data translate into yearly savings of $11,133 and $12,658 for a 2-MGD facility.

The above savings are due largely to staging. The difference between the two values, $1,525/yr, is the savings gained by enzyme addition, and is only 13.7 percent of the $11,133/yr savings for the control system, which is due to staging. Since the savings due to enzyme addition are highly dependant on the cost of enzyme, it is useful to point out that the upper boundary savings from enzyme addition is $4,810/yr. This value could be approached if the cost for enzymes were to decrease.

In order to obtain an indication of relative importance for these savings, two estimates were made for the cost of treatment for a 2-MGD facility. First, a total cost for sewerage was estimated from user charges. The following values were assumed:

Sewage production:	100 gal/capita, day
Average family size:	5 persons
Average yearly sewerage charges per family:	$100

The assumptions result in $400,000/yr for total revenue from user charges. The savings due to staging represent 2.78 percent of the charges, and the savings due to enzyme addition represent only 0.381 percent of the charges.

Next, an estimate for the total cost of anaerobic digestion was made using a recently published Handbook on Estimating Sludge Management Costs (43). The following assumptions were made:

Primary sludge flow:	2950 gal/MG plant flow (44)
Solids concentration:	2 percent
Interest rate:	8.375 percent
Equipment lifetime:	20 yr
Construction period:	2 yr
Cost basis:	last quarter, 1984

TABLE 40. ECONOMIC ASSESSMENT OF CELLULASE PRETREATMENT PROCESS CONCEPT

Item	Xenia Glady Run facility		Scaled-up cellulase control system		Scaled-up cellulase test system	
	$/10^3 gal PS*	$/10^6 gal MWW	$/10^3 gal PS	$/10^6 gal MWW	$/10^3 gal PS	$/10^6 gal MWW
Solids dewatering†	(2.00)	(6.01)	(1.42)	(4.25)	(1.23)	(3.68)
Solids disposal§	(4.00)	(12.01)	(2.83)	(8.49)	(2.45)	(7.35)
Enzyme**	--	--	--	--	(1.49)	(4.50)
CH_4 credit††	7.50	22.49	10.82	32.46	12.45	37.34
Total	1.50	4.47	6.57	19.72	7.28	21.81
Net Savings§§	--	--	5.07	15.25	5.78	17.34

Notes:

* PS = primary sludge.
† Solids dewatering at $20/ton solids.
§ Solids disposal at $8/ton, 20 percent solids.
** Enzyme at $2.25/lb, loading at 2900 ppm. (wt enzyme/wt primary sludge TS) for 24 hr.
†† Methane credit at $5.50/$10^6$ Btu, 10^3 Btu/ft^3 CH_4.
§§ Net savings = Total - Xenia Baseline facility.

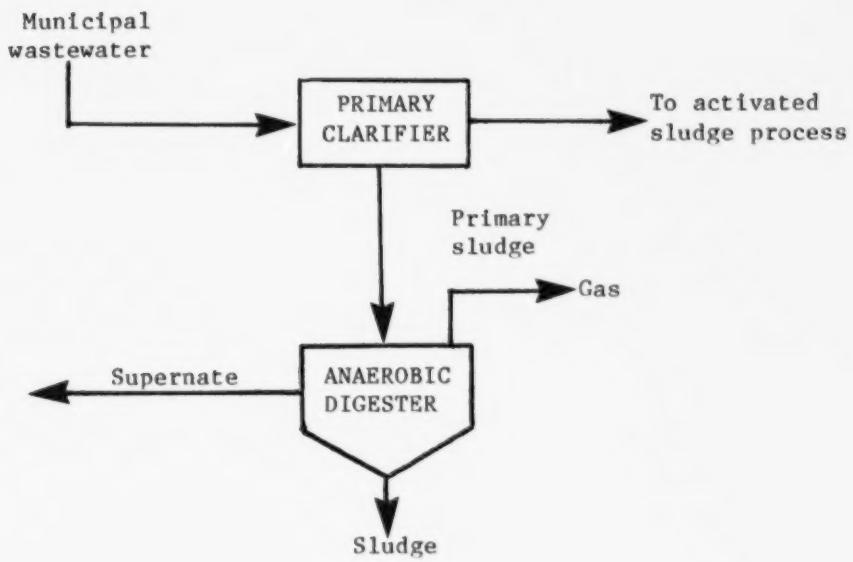
The total estimated annual cost (O&M + annulized capital + contingiencies) for anaerobic digestion is $111,700. The savings are 9.97 percent and 1.36 percent of total digestion costs, respectively, for staging and enzyme addition.

LIPASE SIMULTANEOUS TREATMENT PROCESS CONCEPT

Technical Evaluation

Figure 43 illustrates the simplified Glady Run schematic in comparison to the lipase simultaneous treatment process concept. The addition of lipase enzyme represents the major difference between the systems. Table 41 presents the technical comparison of data obtained from the control and the test systems. The data shown have been directly scaled up from the experimental data described in this report, using a mass ratio basis. As in the cellulase

GLADY RUN ANAEROBIC DIGESTER



GLADY RUN ANAEROBIC DIGESTER
WITH SIMULTANEOUS ENZYME TREATMENT

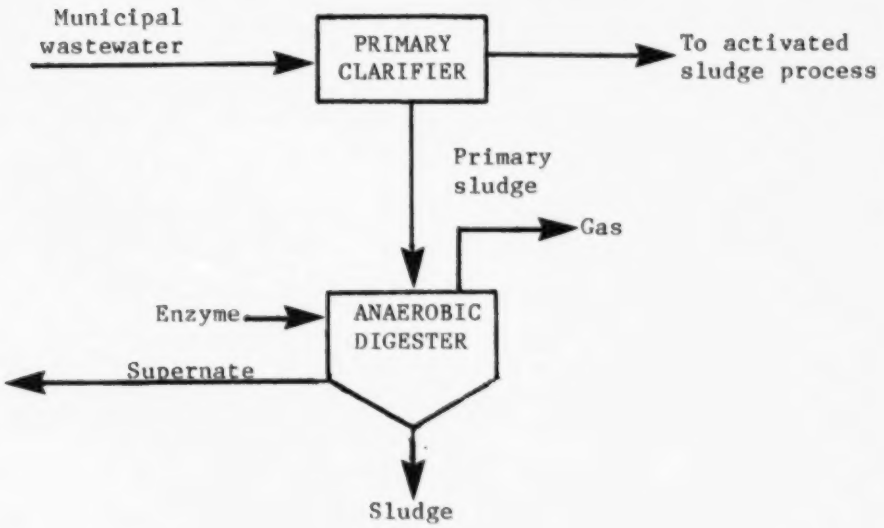


Figure 43. Current and lipase-augmented Glady Run anaerobic digestion configurations.

pretreatment system, total solids output from the digesters has been determined by difference from the input total solids and measured gas production. All results are reported on the basis of primary sludge input.

TABLE 41. SCALED-UP LIPASE PROCESS DATA SUMMARY

Item*	System		
	Scaled-up lipase control system	Scaled-up lipase test system	Optimized oil and grease system
lb digested sludge/ lb primary sludge	.53	.52	.38
ft^3 gas produced/ lb primary sludge	7.07	7.28	9.32
ft^3 CH_4 produced/ lb primary sludge	4.71	4.93	6.30
ft^3 CO_2 produced/ lb primary sludge	2.36	2.35	3.01

* Each item has been normalized to an input of 1 lb primary sludge solids.

Enzyme loadings for the scaled-up test system are difficult to establish because data on the activity and stability of the enzyme at long retention time in the anaerobic digester environment were not obtained in this program. Pending future confirmation of these data, it has been assumed that Figure 34 can be extrapolated to a 15-day retention time and that the enzyme is stable for this time period. Loadings have therefore been set at 1000 ppm (wt enzyme/wt saponifiable oil and grease), sufficient in theory to hydrolyze all of the oil and grease remaining. This loading assumes the literature value of 50 percent destruction of oil and grease in normal anaerobic digestion (4).

Also shown for comparison purposes in Table 41 are the predicted results for an optimized lipase treatment system with gas production based on the possible stoichiometric yield from complete conversion of lipids to gas. The predicted performance data have been calculated from experimental data described in Section 5 of this report and reference values on stiochiometric yields of biogas (25, 40). Enzyme loading for this system is the same as that specified for the scaled-up lipase test system.

The data illustrated in Table 41 indicate that total solids reductions (digested/primary sludge basis) of 47 and 48 percent were observed for the scaled-up lipase control and test systems. The optimized system shown in Table 41 has a predicted solids reduction of 62 percent, considerably higher than any of the other data shown. A similar comparison between cubic feet of methane produced/lb primary sludge shows an increase of 5 percent for the scaled-up test system over the control system data. The optimized system predicts an increase of 34 percent. Using values of 7490 Btu/lb primary sludge and 1000 Btu/ft^3, calculated feed energy recoveries for the scaled-up control, test, and predicted systems are 63, 66, and 84 percent.

Economic Evaluation

The economic analysis of applying a simultaneous lipase treatment/ anaerobic digestion at the Glady Run facility is based on the process flow diagram and scaled-up operating data shown in Figure 43 and Table 41. As in the cellulose pretreatment analysis, the major focus is on solids reduction and methane production. The same method of analyzing cost data has been applied. An enzyme loading of 1000 ppm (wt enzyme/wt saponifiable oil and grease) for 15 days at 35°C has been assumed.

Table 42 illustrates the economic assessment of the scaled-up lipase control and test systems. A net savings per million gallon wastewater of $0.96 is shown for active enzyme-augmented system. These data translate into a yearly savings of $701 for a 2-MGD facility. This is only 0.628 percent of the estimated digestion costs and 0.175 percent of the estimated user charges that were employed before for comparing the cellulase results. Data not shown on this table indicate net savings of $12.58 per million gallon wastewater and $9,183 per year for the predicted optimum facility.

TABLE 42. ECONOMIC ASSESSMENT OF LIPASE SIMULTANEOUS TREATMENT PROCESS CONCEPT

Item	Scaled-up control lipase system		Scaled-up lipase test system	
	$/10^3 gal PS*	$/10^6 gal MWW	$/10^3 gal PS	$/10^6 gal MWW
Solids dewatering†	(1.76)	(5.28)	(1.73)	(5.20)
Solids disposal§	(3.52)	(10.55)	(3.46)	(10.39)
Enzyme**	--	--	(.17)	(.50)
Ch_4 credit††	8.64	25.93	9.05	27.15
Total	3.36	10.10	3.69	11.06
Net Savings§§	--	--	0.33	0.96

Notes:

* PS = primary sludge.
† Solids dewatering at $20/ton solids.
§ Solids disposal at $8/ton, 20 percent solids.
** Enzyme at $2.25/lb, loading at 2900 ppm. (wt enzyme/wt primary sludge TS) for 24 hr.
†† Methane credit at $5.50/$10^6$ Btu, 10^3 Btu/ft^3 CH_4.
§§ Net savings = Total - Xenia Baseline facility.

SECTION 7

CONCLUSIONS

(1) Two-phase digestion used to accommodate cellulase addition in this study shows promise for increasing the efficiency for anaerobic digestion.

(2) The addition of cellulase enzymes shows a moderate improvement over the two-phase digestion process without enzyme addition.

(3) The addition of lipase enzyme under the condition studied shows only a minimal improvement in anaerobic digestion.

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APPENDIX A

EXPERIMENTAL METHODS

Specific aspects of many of the procedures employed in this program have been described in the text of this report. This section will review those areas not discussed previously.

COLLECTION OF SAMPLES FROM THE GLADY RUN FACILITY

Sampling sites at the Glady Run facility were chosen to provide representative samples that could be collected quickly and without unnecessary interruption of normal plant operations. Primary sludge was collected from the transfer line carrying sludge from the primary tank into the digester. Digested sludge was collected from the top access ports of the digester lid and from a recirculating pump located near the base of the digester. All samples were collected in clean 1-gal polyethylene bottles and stored at 4°C prior to use.

MEASUREMENT METHODS AND TEST PROCEDURES

The following measurements and tests were performed by SYSTECH according to the methods outlined in Standard Methods for the Examination of Water and Wastewater, 15th edition, APHA-AWWA-WPCP, 1980:

1.	Total solids	Method 209A
2.	Total volatile solids	Method 209E
3.	Kjeldahl nitrogen	Methods 420B and 417D
4.	Total phosphate	Methods 424 and 424E
5.	pH	Method 423
6.	Oil and grease	Method 503D
7.	Volatile acids	Method 504A
8.	Alkalinity	Method 403

BOD analyses were performed by the City of Xenia laboratories using Method 507.

In addition, a number of procedures from other sources were performed by SYSTECH in this study. These include the following:

1. Heat content - ASTM D2015-66: "Gross Calorific Value of Solid Fuel by the Adiabatic Bomb Calorimeter."

2. Cellulose - EPA 6700-73-01: "Gravimetric Method for Determining Cellulose," Richard D. Lossin, modified by substitution of toluene and methanol for benzene and ether.

3. Specific resistance and filter yield - P. Aarne Vesilind, Treatment and Disposal of Wastewater Sludges, 1974.

4. Gas composition - Gas samples were analyzed for CO_2 and CH_4 by gas chromatography employing the following conditions:

   Column: 7 ft × 1/8 in. SS with Carbosieve S, 100/120 mesh.
   Column temperature: 150°C isothermal
   Flow rate: 60 ml/min
   Detector: Thermal conductivity, 175 mA
   Sample Size: 100 µl
   Instrument: Varian Model 1400

   Quantitation was accomplished by comparing peak area response to calibration curves prepared from known standards of CO_2 and CH_4 in the 100, 50, and 25 percent volume range.

5. Enzyme assay procedure and reducing sugar analysis - Modified from "Cellulase Activity Determination," NOVO Laboratories, Inc., 142a-GB, 1976.

   Part 1: Activity Assay Procedure

   A. Suspend 1.000-g Avicel powder in 100-mL 0.1 molar KHP pH 4.8.

   B. For each enzyme solution to be tested, set up replicate groups of two 13 × 100-mm screw-top tubes.

      To each tube add: 1.50 mL of Avicel suspension (15 mg substrate).

      To one tube (sample blank) add: 1.00 mL STOP reagent (.11 molar Na_2HPO_4, pH 12.5, 10.0-g $Na_2HPO_4.2H_2O$ and 10.0-g NaOH to 1 L in demineralized water).

      To one tube (assay tube) add: 0.50 mL enzyme containing solution.

   C. Place tubes in preheated 50°C water bath in shaker and incubate 20 min with constant shaking at 2-Hz, 2-cm displacement.

   D. At end of incubation, add 1 mL STOP reagent to the assay tubes thereby stopping enzyme reaction. Add 0.50 mL enzyme containing solution to the sample blank.

   Part 2: Ferricyanide Method for Determination of Reducing Sugars

Note: Prepare glucose standards of 0, 20, 40, 60, 80, and 100 µg/ml and carry them through the following colorimetric analysis procedure.

E. Remove an aliquot from tubes at Step 4 and dilute to 2.0 mL with demineralized H_2O such that glucose concentration is 20 to 100 µg/mL. Add to this 1 mL STOP reagent.

F. Add 1.5 mL $K_3Fe(CN)_6$ solution [1.16-g $K_3Fe(CN)_6$, 15.06-g $Na_2HPO_4.7\ H_2O$, 2.2-g NaOH to 1 L in demineralized water] to this 3-mL volume in a 13 × 100-mm test tube.

G. Boil tubes for 5 minutes at 100°C. Then hold for 10 min at 25°C.

8. Record absorbance at 420 nm.

6. Saponification value - Plummer, D. T., An Introduction to Practical Biochemistry, 1971.

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APPENDIX B

QA/QC PROGRAM AND DATA SUMMARIES

INTENDED USE OF DATA AND DATA QUALITY ACCEPTANCE CRITERIA

The overall objective of this program was to examine the potential of cellulose and oil/grease specific enzymatic hydrolysis to enhance the efficiency of anaerobic digestion of municipal wastewater sludges. All data collected in this program were used toward this end. The final acceptance criteria in this project were determined by a statistical test of the available data to determine whether cellulose specific enzymes significantly enhance anaerobic digestion of MWW sludges. Data were judged as acceptable if they permit the performance of this test.

PROJECT REQUIREMENTS FOR PRECISION, ACCURACY, REPRESENTIVENESS COMPLETENESS AND COMPARABILITY

As noted previously, the overall quality acceptance criteria was based on assuring that the data would permit a statistically valid conclusion regarding the success of the proposed technology. In order to meet this criteria, all measurement methods were carefully documented to provide data on precision, accuracy, representativeness, completeness, and comparability. This was accomplished during the course of the program by pursuing standard QA/QC techniques as described in the following items.

PROCEDURES FOR SELECTION OF SITES AND COLLECTION OF SAMPLES

This program required the collection of samples of primary and digested sludge from the Xenia treatment plant. Sampling sites were chosen to provide representative samples that could be collected quickly and without unnecessary interruption of normal plant operations. Primary sludge was collected from the transfer line carrying sludge form the primary into the digester. Digested sludge was collected either from the top access ports of the digester lid or from a recirculating pump located near the base of the digester. All samples were collected in clean 1-gal polyethlene bottles. In the case of digested sludge samples collected from the top access ports, a sampling thief was employed to collect and then transfer samples to a polyethylene bottle. Samples were also collected from the lab-scale experiments, digesters, and enzyme reactors operated at SYSTECH's laboratory facilities. These samples were normally collected in 10- to 400-mL volumes using clean laboratory glassware.

SAMPLE HANDLING, IDENTIFICATION PRESERVATION, TRANSPORTATION, AND STORAGE

All samples collected from the Xenia plant were transported immediately to either the Xenia Municipal Wastewater Laboratory or SYSTECH Laboratory. Analyses were conducted as soon as possible, with interim storage of samples at 4°C. No other preservation methods were employed. Samples destined for analysis by SYSTECH were identified by attachment of the following label.

Plant _____ City _____
Site _____ Sample Type _____
Date _____ Run Number _____
Contents _____
Log Number _____ Level Marked _____
VOLUME : Initial _____ Final _____
Sample by _____ Cleanup by _____
REMARKS - _____
_____
SYSTECH®

MEASUREMENT METHODS AND TEST PROCEDURES

Analyses of samples for BOD were made by the City of Xenia personnel. All methods are those outlined in Standard Methods for the Examination of Water and Wastewater, 15th edition, APHA-AWWA-WPCF.

SYSTECH performed the following measurements in this program. All procedures, unless otherwise noted, are from Standard Methods.

1. Total Volatile and Fixed Residue
2. pH Value - Potentiometric Method
3. Alkalinity
4. Chromatographic Separation Method for Organic Acids
5. Enzyme Activity - Appendix A
6. Gas Composition - Appendix A
7. Heat Content - ASTM D2015-66, Gross Calorific Value of Solid Fuel by the Adiabatic Bomb Calorimeter
8. TKN
9. Total Phosphorus
10. Glucose
11. Cellulose - EPA 6700-73-01, Gravimetric Method for Determining Cellulose
12. Solids Capture, P. Aarne Vesilind, Treatment and Disposal of Wastewater Sludges, 1974
13. Specific Resistance - Vesilind, as above

QA/QC PROCEDURES

QA/QC was implemented as outlined in the EPA 600/4-79-019. Field and laboratory sample replicates and split sample were analyzed at a frequency of 5 percent of the normal analytical load. Laboratory QA samples were run at a frequency of 10 percent of the normal analytical load. These included replicates as well as spiked samples. Standard quality control procedures such as control charts for accuracy and precision were used as much as possible.

DATA REDUCTION AND REPORTING PROCEDURES

All data reductions were subject to verification by an independent in-house analyst. The majority of the experiments conducted are factorial designs, and the significance of these data were tested by standard statistical techniques for analysis of variance of 2 and 3 factor designs such as those detailed in Experimental Statistics, U.S. Department of Commerce Handbook No. 91 and Probability and Statistics for Engineers and Scientists, Ronald E. Walpole and Raymond H. Myers, 1972. In Phase 3 of the project, the results of the laboratory-scale, process-lines studies were tested by comparing the mean performance of the enzyme treatment systems with a control system.

DATA SUMMARIES

Tables B-1 through B-12 summarize all of the data obtained in the Pretreatment and Posttreatment Cellulase Studies and the Simultaneous Treatment Lipase Study.

TABLE B-1. PRETREATMENT LAB SCALE STUDY: PRIMARY SLUDGE DATA

Day	TS (%)	VS (%)	pH
1	-	-	5.5
2	-	-	5.4
3	2.46	80.0	5.4
3	2.22	86.4	5.1
4	2.84	80.0	5.0
5	2.37	90.9	5.0
7	2.65	84.2	-
8	3.11	84.4	-
9	2.24	95.5	5.8
10	2.59	79.9	5.5
11	2.35	81.7	5.4
12	2.48	81.2	-
13	-	-	-
14	-	-	-
15	3.19	85.7	5.8
16	-	-	-
17	2.67	85.3	-
18	2.28	84.7	5.6
19	2.69	85.6	-
20	2.69	85.6	-
21	2.69	85.6	-

TABLE B-2. PRETREATMENT LAB SCALE STUDY: REACTOR EFFLUENT DATA

	Control System					Test System				
Day	TS %	VS %	pH	VA mg/L	Cellulose % of TS	TS %	VS %	pH	VA mg/L	Cellulose % of TS
1	2.32	80.0	5.2			2.32	80.0	5.2		
2	2.17	80.9	5.2			1.96	78.9	5.1		
3	2.13	77.3	5.0	2891	14.98	1.86	95.0	5.0	2869	17.19
4	2.28	86.4	4.9			2.43	80.9	4.9		
5	2.58	90.0	4.9			2.90	77.3	4.9		
5d	2.50	-	-	5760	13.69	2.55	81.3	4.9	4715	9.65
6	2.94	87.5	4.9			2.88	79.2	4.9		
7	2.21	83.0	5.4			2.50	83.3	5.4		
8	2.64	96.2	5.2	4626	22.10	2.29	90.5	5.1	4582	20.3
19	-	-	6.0			-	-	5.6		
10	2.54	77.6	5.5			2.33	82.1	5.4		
11	-	-	5.5	3781	21.90	-	-	5.3	6005	18.1
12	2.23	80.5	5.3			2.20	80.3	5.1		
13	2.51	80.4	5.2			2.52	80.9	5.2		
14	2.52	80.5	5.3	3959	15.65	2.56	80.8	5.2	5560	11.72
15	2.61	80.5	5.3			2.58	81.0	5.3		
16	3.45	85.0	5.4			2.39	84.9	5.3		
17	2.84	84.1	5.5	4960	20.85	2.39	84.9	5.4	4782	18.2
18	2.58	84.3	5.4			2.70	84.6	5.3		
29	2.33	84.2	5.7			2.29	83.9	5.5		
20	2.46	84.3	5.5	4330		2.37	83.7	5.2	4752	
21	2.56	84.6	5.3			2.55	84.5	5.2		

TABLE B-3. PRETREATMENT LAB SCALE STUDY: CONTROL SYSTEM DIGESTER DATA

Day	pH	Gas Vol mL	CH_4 %	CO_2 %	VA mg/L	Alk mg/L	TKN mgN/L	TOT P mgP/L	BOD mg/L	Btu/lb (filter cake)	SR*	FY†
1	6.9	5381	64.86	35.14	267	2750	–	–	2075	7142	–	–
2	7.0	3483	68.68	31.32	–	3150	–	–	–	–	–	–
3	7.0	4091	68.49	31.51	–	–	1504	359	1725	–	28.1	.444
4	6.9	3981	67.35	32.65	–	–	–	–	–	–	–	–
5	6.9	3711	67.24	32.76	–	–	–	–	1963	–	–	–
6	7.0	3926	–	–	–	–	1212	550	–	–	17.76	.444
7	7.2	3649	–	–	267	–	–	–	–	7075	–	–
8	6.9	3607	68.85	31.15	156	2900	–	–	1838	–	–	–
9	6.8	3855	67.28	32.72	–	2750	1760	273	–	–	22.1	.326
10	6.9	3672	67.66	32.34	–	–	–	–	1088	–	–	–
11	6.9	3602	68.24	31.76	–	–	–	–	–	–	–	–
12	6.9	3598	67.28	32.72	–	–	1182	273	1350	–	18.42	.444
13	6.9	3631	–	–	–	–	–	–	–	–	–	–
14	6.9	3841	–	–	156	2450	–	–	–	6796	–	–
15	6.9	4061	68.08	31.92	89	2150	1086	207	1938	–	10.58	.474
16	6.9	3503	68.75	31.25	–	–	–	–	–	–	–	–
17	6.9	4717	64.84	35.16	–	–	–	–	963	–	–	–
18	6.9	5004	63.73	36.27	–	–	957	326	–	–	19.52	.592
19	6.9	4647	64.23	35.77	–	–	–	–	1825	–	–	–
20	6.9	4582	–	–	–	2450	–	–	–	–	–	–
21	6.9	4173	–	–	89	2500	922	357	1425	6339	–	–

* Specific resistance.

† Filter yield.

TABLE B-4. PRETREATMENT LAB SCALE STUDY: TEST SYSTEM DIGESTER DATA

Day	pH	Gas Vol mL	CH_4 %	CO_2 %	VA mg/L	Alk mg/L	TKN mgN/L	TOT P mgP/L	BOD mg/L	Btu/lb (filter cake)	SR*	FY†
1	6.9	6687	60.85	39.15	67	3100	-	-	2463	6455	-	-
2	7.0	5393	67.89	32.11	-	3200	-	-	-	-	-	-
3	7.0	5068	68.49	31.51	-	-	1638	345	2125	-	27.88	.533
4	7.0	4933	67.14	32.86	-	-	-	-	-	-	-	-
5	7.0	4167	68.83	31.17	-	-	-	-	1713	-	-	-
6	7.0	4268	-	-	-	-	1446	314	-	-	23.24	.533
7	7.2	3934	-	-	67	-	-	-	-	7136	-	-
8	7.0	3798	70.06	29.94	67	3200	-	-	1238	-	-	-
9	6.9	3994	68.59	31.41	-	3000	1327	259	-	-	17.82	.385
10	7.0	3803	69.30	30.70	-	-	-	-	638	-	-	-
11	6.9	3585	68.66	31.34	-	-	-	-	-	-	-	-
12	6.9	3598	69.05	30.95	-	-	983	222	1113	-	13.17	.355
13	6.9	3796	-	-	-	-	-	-	-	-	-	-
14	7.0	3605	-	-	67	-	-	-	-	6838	-	-
15	6.9	3940	70.44	29.56	423	2650	932	326	888	-	12.52	.474
16	6.9	3399	70.91	29.09	-	2550	-	-	-	-	-	-
17	6.9	4963	65.38	34.62	-	-	-	-	1850	-	-	-
18	6.9	4514	65.45	34.55	-	-	1010	242	-	-	10.93	.533
19	6.9	4404	66.04	33.96	-	-	-	-	1925	-	-	-
20	6.9	5170	-	-	-	2500	-	-	-	-	-	-
21	6.9	4440	-	-	423	2500	730	284	1865	6399	-	-

* Specific resistance.
† Filter yield.

TABLE B-5. POSTTREATMENT LAB SCALE STUDY: PRIMARY SLUDGE DATA

Day	TS %	VS %	pH
1	-	-	-
2	2.88	73.67	5.2
3	2.31	76.92	5.5
4	2.35	77.83	5.5
5	2.40	78.15	5.5
6	2.64	78.29	5.5
7	-	-	5.2
8	2.39	77.40	5.2
9	2.31	75.30	5.1
10	2.41	76.62	5.0
11	2.40	77.02	5.0
12	2.53	76.63	5.0
13	2.70	77.12	5.0
14	-	-	5.0
15	3.41	80.98	5.2
16	3.53	80.32	5.3
17	3.17	80.10	5.2
18	3.21	82.91	5.1
19	2.13	83.13	5.2
20	3.09	83.00	5.1
21	2.25	82.23	5.3
22	-	-	-
23	2.52	82.24	5.8
24	2.42	82.67	5.8
25	2.62	83.19	5.7
26	-	-	-
27	2.62	82.82	5.8
28	2.39	80.93	5.8
29	2.56	82.63	5.8
30	2.66	78.81	5.6
31	2.60	79.36	5.8
32	2.56	79.34	5.8
33	2.48	80.81	5.5
34	2.48	80.89	5.5
35	2.48	81.11	5.8
36	2.62	80.99	5.8
37	2.53	81.00	5.8
38	2.23	66.9	5.8
39	2.47	66.5	5.9
40	2.32	67.45	5.9
41	2.38	64.82	5.6
42	2.37	66.00	6.1
43	2.45	63.46	6.1
44	2.49	64.56	6.1
45	2.37	67.02	6.0
46	2.55	65.15	5.9
47	3.28	62.81	6.1
48	2.53	81.13	5.7

TABLE B-6. POSTTREATMENT LAB SCALE STUDY: CONTROL SYSTEM REACTOR EFFLUENT DATA

Day	TS %	VS %	pH
1	1.26	56.58	5.5
2	1.84	58.35	6.0
3	-	-	6.1
4	1.93	57.51	6.2
5	-	-	5.9
6	1.96	59.99	6.0
7	-	-	5.6
8	-	-	5.7
9	1.60	56.38	5.8
10	-	-	5.7
11	2.15	61.16	5.5
12	-	-	5.5
13	1.78	59.30	5.8
14	-	-	5.2
15	-	-	-
16	1.86	62.78	5.8
17	-	-	5.7
18	2.05	61.45	5.4
19	-	-	5.5
20	1.98	59.74	6.1
21	-	-	6.1
22	-	-	5.0
23	1.81	65.42	5.8
24	-	-	5.6
25	1.92	61.66	5.3
26	-	-	5.8
27	1.66	65.94	5.5
28	-	-	6.0
29	-	-	5.9
30	1.88	66.33	5.9
31	-	-	5.2
32	1.70	67.55	5.6
33	-	-	5.6
34	1.74	67.58	5.6
35	-	-	5.5
36	-	-	5.5
37	1.63	66.21	5.5
38	-	-	5.6
39	1.58	65.25	5.4
40	1.95	68.24	5.2
41	1.67	64.03	5.3
42	-	-	5.8
43	-	-	5.8
44	1.62	64.59	5.8
45	-	-	5.8
46	1.80	59.04	5.7
47	-	-	5.6
48	1.66	62.78	5.5

TABLE B-7. POSTTREATMENT LAB SCALE STUDY: TEST SYSTEM REACTOR EFFLUENT

Day	TS %	VS %	pH
1	1.53	57.32	5.8
2	1.39	56.46	5.6
3	-	-	5.7
4	1.94	56.60	5.9
5	-	-	5.4
6	1.80	59.12	5.4
7	-	-	5.8
8	-	-	5.8
9	1.71	53.92	5.7
10	-	-	5.9
11	1.88	59.15	5.5
12	-	-	5.5
13	2.31	64.31	5.7
14	-	-	5.2
15	-	-	-
16	2.08	59.74	5.7
17	-	-	5.7
18	1.94	62.02	5.3
19	-	-	6.0
20	1.90	62.39	5.7
21	-	-	5.8
22	-	-	4.5
23	1.83	61.60	6.1
24	-	-	5.6
25	2.02	65.48	5.2
26	-	-	5.7
27	1.61	64.22	5.5
28	-	-	6.1
29	-	-	5.8
30	1.46	64.48	5.9
31	-	-	5.2
32	1.62	66.44	5.6
33	-	-	5.6
34	1.74	67.70	5.6
35	-	-	5.5
36	-	-	5.5
37	1.76	67.65	5.5
38	-	-	5.5
39	1.43	64.49	5.6
40	1.75	64.63	5.4
41	1.64	62.92	5.2
42	-	-	5.3
43	-	-	5.8
44	1.60	64.85	5.8
45	-	-	5.8
46	1.66	60.63	5.7
47	-	-	5.6
48	1.65	60.64	5.5

TABLE B-8. POSTTREATMENT LAB SCALE STUDY: CONTROL SYSTEM DIGESTER EFFLUENT DATA

Day	TS %	VS %	pH	Gas Vol mL	CH_4 %	CO_2 %	VA mg/L	Alk mg/L	TKN mgN/L	TOT P mg/P/L	BOD mg/L	FY*	SR†	Btu/lb (filter cake)
1	2.01	55.57	6.9	1805	-	-	-	-		-	-	-	-	-
2	1.80	57.86	6.9	1877	55.49	44.51	-	-	-	-	-	-	-	-
3	1.80	57.97	7.0	1463	75.85	24.15	410	2200	-	138	3225	-	-	-
4	1.75	57.72	7.0	1713	74.99	25.01	-	-	867	-	-	-	-	-
5	1.81	59.74	6.9	1693	72.99	27.01	410	2300	-	-	-	-	-	-
6	1.76	59.33	6.9	1587	69.49	30.51	-	-	-	-	-	-	-	-
7	1.60	57.57	6.8	1271	-	-	-	-	-	-	-	-	-	-
8	1.64	56.21	6.8	1675	-	-	-	-	-	-	2550	414	10.39	6594
9	1.55	58.60	6.9	1545	76.13	23.87	-	-	-	-	-	-	6.49	-
10	1.61	59.25	6.8	1575	76.30	23.70	338	2300	-	83	-	-	-	-
11	1.70	58.76	6.8	1377	76.08	23.92	-	-	805	-	-	-	-	-
12	1.78	62.88	6.8	1484	78.81	21.19	338	2400	-	-	-	-	-	-
13	1.67	62.14	6.9	1566	74.60	25.40	-	-	-	-	-	-	-	-
14	1.62	63.53	6.8	1758	-	-	-	-	-	-	-	-	-	-
15	1.81	65.55	6.8	1008	-	-	-	-	-	-	2475	533	11.25	6971
16	1.69	65.60	6.8	1942	74.49	25.51	-	-	-	-	-	-	13.25	-
17	1.91	68.08	6.7	1509	76.37	23.63	423	2250	-	187	-	-	-	-
18	1.80	65.49	6.8	1915	74.33	25.67	-	-	802	-	-	-	-	-
19	1.82	66.60	6.9	1682	72.31	27.69	266	-	-	-	-	-	-	-
20	1.76	64.61	6.8	1867	70.86	29.14	-	-	-	-	-	-	-	-
21	1.83	68.02	6.8	1898	-	-	-	-	-	-	-	-	-	-
22	1.69	67.21	6.8	1822	-	-	-	-	-	-	2775	651	28.02	7808
23	1.61	64.69	6.9	1546	68.72	31.28	-	-	-	-	-	-	29.96	-
24	1.63	65.48	6.9	1644	71.72	28.28	842	2150	-	263	-	-	-	-
25	1.53	65.21	6.8	1640	69.41	30.59	-	-	757	-	-	-	-	-
26	1.64	66.54	6.8	1624	68.05	31.95	626	2100	-	-	-	-	-	-
27	1.52	65.64	7.0	1635	66.89	33.11	-	-	-	-	-	-	-	-
28	1.53	65.97	6.8	1235	-	-	-	-	-	-	-	-	-	-
29	1.56	65.93	6.8	1639	-	-	-	-	-	-	2975	533	33.44	7727
30	1.52	67.14	6.9	1587	66.41	33.59	-	-	-	-	-	-	32.78	-
31	1.51	66.56	6.8	1614	67.00	33.00	146	1700	-	149	-	-	-	-
32	1.53	69.44	6.8	1564	66.53	33.47	-	-	716	-	-	-	-	-
33	1.47	66.31	6.9	1405	70.52	29.48	74	2400	-	-	-	-	-	-
34	1.44	66.61	6.9	1649	67.92	32.08	-	-	-	-	-	-	-	-
35	1.51	67.15	6.9	1294	-	-	-	-	-	-	3225	-	-	-
36	1.51	67.26	7.0	1696	-	-	-	-	-	-	-	562	24.55	8162
37	1.47	66.83	7.0	1909	67.12	32.88	-	-	-	-	-	-	35.13	-
38	1.46	67.48	7.0	-	-	-	146	2200	-	218	-	-	-	-
39	1.60	68.36	7.1	1606	40.53	59.47	-	-	788	-	-	-	-	-
40	1.69	69.71	7.2	1454	35.48	64.52	98	1950	-	-	-	-	-	-
41	1.47	64.04	7.0	1442	40.22	59.78	-	-	-	-	-	-	-	-
42	1.40	62.79	6.9	1132	-	-	-	-	-	-	-	-	-	-
43	1.45	64.35	6.9	1106	-	-	-	-	-	-	1675	592	13.34	7625
44	1.40	62.83	7.0	1207	38.18	61.82	-	-	-	-	-	-	16.65	-
45	1.45	61.27	6.9	1030	40.16	59.84	-	-	-	161	-	-	-	-
46	1.44	62.55	6.9	1140	43.22	56.78	-	-	713	-	-	-	-	-
47	1.47	61.84	7.1	1137	58.58	41.42	-	-	-	-	-	-	-	-
48	1.45	60.86	7.0	1144	53.24	46.76	-	-	-	-	1625	503	14.03	6973
									-					

* Filter yield.
† Specific resistance.

TABLE B-9. POSTTREATMENT LAB SCALE STUDY: TEST SYSTEM DIGESTER EFFLUENT DATA

Day	TS %	VS %	pH	Gas Vol mL	CH_4 %	CO_2 %	VA mg/L	AlK mg/L	TKN mgN/L	TOT P mgP/L	BOD mg/L	FY*	SR†	Btu/lb (filter cake)
1	2.29	59.88	7.0	1032	-	-	-	-	-	-	-	-	-	-
2	2.01	61.84	7.0	995	58.97	41.03	-		-	-	-	-	-	-
3	1.92	61.52	7.0	774	61.88	38.12	770	2000	848	280	5600	-	-	-
4	1.91	61.54	6.9	926	64.69	35.31	-	-	-	-	-	-	-	-
5	1.83	62.23	6.9	1037	63.23	36.77	770	2150	-	-	-	-	-	-
6	1.79	61.58	6.9	1058	65.83	34.17	-	-	-	-	-	-	-	-
7	1.72	58.83	6.8	846	-	-	-	-	-	-	-	-	-	-
8	1.71	59.68	6.8	1140	-	-	-	-	-	-	4000	.414	13.07	5541
9	1.63	58.42	6.9	1099	68.32	31.68	-	-	-	-	-	-	17.68	-
10	1.54	61.00	6.8	1191	66.96	33.04	650	2100	177	265	-	-	-	-
11	1.63	61.54	6.8	1010	69.03	30.97	-	-	-	-	-	-	-	-
12	1.71	63.50	6.8	1074	69.40	30.60	650	2250	-	-	-	-	-	-
13	1.67	61.29	6.5	993	49.99	50.01	-	-	-	-	-	-	-	-
14	1.82	64.17	6.8	928	-	-	-	-	-	-	-	-	-	-
15	1.81	65.73	6.8	311	-	-	-	-	-	-	4175	.444	25.11	7744
16	1.89	66.43	6.9	1232	52.02	47.98	-	-	-	-	-	-	16.29	-
17	1.76	65.91	7.0	816	38.67	61.33	794	2500	793	160	-	-	-	-
18	1.84	67.10	6.7	1047	45.17	54.83	-	-	-	-	-	-	-	-
19	1.98	68.38	6.7	682	51.58	48.42	962	-	-	-	-	-	-	-
20	1.97	68.84	6.8	687	43.27	56.73	-	-	-	-	-	-	-	-
21	1.96	69.82	6.8	628	-	-	-	-	-	-	-	-	-	-
22	1.79	69.27	6.9	730	-	-	-	-	-	-	5400	.592	25.33	8139
23	1.77	67.70	6.8	833	33.43	66.57	-	-	-	-	-	-	18.09	-
24	1.80	69.17	6.8	708	36.94	63.06	2056	1750	733	178	-	-	-	-
25	1.81	68.82	6.8	681	39.08	60.92	-	-	-	-	-	-	-	-
26	1.82	70.98	6.9	723	49.87	50.13	746	1850	-	-	-	-	-	-
27	1.85	66.57	6.9	762	47.94	52.06	-	-	-	-	-	-	-	-
28	1.72	69.63	6.6	658	-	-	-	-	-	-	-	-	-	-
29	1.63	69.16	6.6	912	-	-	-	-	-	-	5700	.562	30.75	8439
30	1.63	70.43	6.7	804	44.36	55.64	-	-	-	-	-	-	30.20	-
31	1.65	70.46	6.6	817	44.23	55.77	674	2250	790	245	-	-	-	-
32	1.46	67.44	6.7	783	51.28	48.72	-	-	-	-	-	-	-	-
33	1.58	69.34	6.8	644	37.61	62.39	1010	2200	-	-	-	-	-	-
34	1.60	70.21	6.8	761	43.65	56.35	-	-	-	-	-	-	-	-
35	1.86	74.04	6.8	566	-	-	-	-	-	-	-	-	-	-
36	1.64	70.39	6.9	767	-	-	-	-	-	-	4900	.503	30.21	8644
37	1.57	70.77	6.9	827	26.48	73.52	-	-	-	-	-	-	31.55	-
38	1.63	71.96	7.0	-	-	-	866	1700	766	161	-	-	-	-
39	1.58	70.06	7.0	748	40.53	59.47	-	-	-	-	-	-	-	-
40	1.57	69.17	7.0	733	35.48	64.52	626	1850	-	-	-	-	-	-
41	1.57	68.75	7.0	566	40.22	59.78	-	-	-	-	-	-	-	-
42	1.58	67.43	6.8	620	-	-	-	-	-	-	-	-	-	-
43	1.60	67.40	6.9	598	-	-	-	-	-	-	6200	.562	26.23	8369
44	1.48	67.26	6.9	660	38.18	61.82	-	-	-	-	-	-	39.61	-
45	1.48	64.92	6.9	651	40.16	59.84	-	-	731	187	-	-	-	-
46	1.50	65.73	6.9	732	43.22	56.78	-	-	-	-	-	-	-	-
47	1.48	64.08	7.0	747	58.58	41.42	-	-	-	-	-	-	29.69	-
48	1.46	63.14	7.0	689	53.24	46.76	-	-	-	-	3525	.473	27.95	7359

* Filter yield.
† Specific resistance.

TABLE B-10. LIPASE STUDY: PRIMARY SLUDGE DATA

Day	TS %	VS %	pH
1	2.39	79.02	5.7
2	2.43	79.50	5.6
3	2.43	78.77	5.7
4	2.56	81.69	5.7
5	2.44	81.99	5.7
6	2.41	81.73	5.7
7	2.42	82.07	5.7
8	2.55	81.97	5.7
9	2.41	82.36	5.7
10	2.38	81.72	5.7
11	2.36	81.90	5.7
12	2.43	81.83	5.8
13	2.44	81.46	5.8
14	2.54	80.75	5.7
15	2.57	81.41	5.6
16	2.54	81.46	5.7
17	2.46	82.41	5.8
18	2.46	82.62	5.8
19	2.45	81.90	5.8
20	2.38	82.21	5.8
21	-	-	-
22	-	-	-
23	2.52	81.10	5.9
24	2.49	80.96	5.9
25	2.39	80.57	5.9
26	2.33	80.23	5.8
27	2.48	81.60	5.7
28	2.35	80.48	5.8
29	1.90	78.46	5.8
30	2.18	77.94	5.9
31	2.45	77.69	5.9
32	2.51	76.91	5.8
33	2.54	77.75	5.8
34	2.81	76.98	5.8
35	2.61	78.37	5.8

TABLE B-11. LIPASE STUDY: CONTROL SYSTEM DIGESTED SLUDGE DATA

Day	TS %	VS %	pH	Gas Vol ml.	CH_4 %	CO_2 %	VA mg/L	AlK mg/L	TKN mgN/L	TOT P mgP/l	BOD mg/L	FY*	SR†	Btu/lb (filter cake)
1	1.41	68.52	7.1	5835	-	-	-	-	-	-	-	-	-	-
2	1.44	66.99	7.1	2355	-	-	-		-	-	-	-	-	-
3	1.53	66.02	7.4	2623	70.08	29.92	96	2750	-	-	1725	-	-	-
4	1.51	66.91	7.3	2960	66.70	33.30	-	-	-	-	-	-	-	-
5	1.43	68.97	7.4	2962	69.38	30.62	48	3000	-	-	-	-	-	-
6	1.40	68.52	7.0	3052	-	-	-	-	-	-	-	-	-	-
7	1.42	69.58	7.0	3064	-	-	-	-	226	46	1900	11.9	44.6	6703
8	1.47	66.66	7.3	3548	63.14	36.86	48	3275	-	-	-	-	-	-
9	1.49	67.42	7.1	3291	69.17	30.83	-	-	-	-	-	-	-	-
10	1.54	67.80	7.2	3153	69.78	30.22	48	3050	-	-	-	-	-	-
11	1.44	67.87	7.1	3362	68.48	31.52	-	-	-	-	-	-	-	-
12	1.45	67.79	7.1	3214	-	-	60	2750	-	-	-	-	-	-
13	1.47	67.93	7.0	3294	69.18	30.82	-	-	-	-	-	-	-	-
14	1.45	68.10	7.0	3427	-	-	-	-	265	ND	1700	5.3	29.3	7484
15	1.46	68.12	7.2	3405	-	-	96	3150	-	-	-	-	-	-
16	1.52	68.90	7.1	3231	72.53	27.47	-	-	-	-	-	-	-	-
17	1.52	68.59	7.1	3019	70.82	29.18	96	2925	-	-	-	-	-	-
18	1.48	68.32	7.1	3358	69.90	30.10	-	-	-	-	-	-	-	-
19	1.56	66.16	7.2	3295	69.97	30.03	48	2350	-	-	-	-	-	-
20	1.45	68.05	7.0	2939	-	-	-	-	-	-	-	-	-	-
21	1.47	68.95	7.0	3192	-	-	-	-	394	16	1800	7.1	29.9	7916
22	1.48	68.32	7.2	3146	70.64	29.36	24	2450	-	-	-	-	-	-
23	1.44	69.30	7.1	3533	70.03	29.97	-	-	-	-	-	-	-	-
24	1.49	67.99	7.2	2984	71.18	28.82	72	2400	-	-	-	-	-	-
25	1.60	68.62	7.0	3075	71.34	28.66	-	-	-	-	-	-	-	-
26	1.42	68.32	7.1	2901	71.34	28.66	24	2500	-	-	-	-	-	-
27	1.58	69.50	7.0	3024	-	-	-	-	-	-	-	-	-	-
28	1.63	69.95	7.0	2835	-	-	-	-	237	16	2050	6.0	22.1	7225
29	1.39	68.12	6.9	2824	67.36	32.64	72	2500	-	-	-	-	-	-
30	1.42	68.28	6.9	1679	72.47	27.53	-	-	-	-	-	-	-	-
31	1.36	66.39	7.0	2173	72.01	27.99	48	2500	-	-	-	-	-	-
32	1.37	67.97	6.9	2580	69.73	30.27	-	-	-	-	2300	-	-	-
33	1.44	67.20	7.0	2644	71.37	28.63	48	2050	-	-	-	-	-	-
34	1.35	67.02	6.9	4490	-	-	-	-	-	-	-	-	-	-
35	1.53	63.71	7.0	2600	66.91	33.09	72	2400	220	16	2250	9.2	22.0	7438

* Filter yield.
† Specific resistance.

TABLE B-12. LIPASE STUDY: TEST SYSTEM DIGESTED SLUDGE DATA

Day	TS %	VS %	pH	Gas Vol mL	CH_4 %	CO_2 %	VA mg/L	AlK mg/L	TKN mgN/L	TOT P mgP/L	BOD mg/L	FY*	SR*	Btu/lb (filter cake)
1	1.37	66.79	7.2	5788	-	-	-	-	-	-	-	-	-	-
2	1.49	66.40	7.2	2422	-	-	-		-	-	-	-	-	-
3	1.54	66.52	7.4	2675	70.39	29.61	408	3000	-	-	1600	-	-	-
4	1.50	67.29	7.1	3132	67.87	32.13	-	-	-	-	-	-	-	-
5	1.43	69.96	7.4	3066	70.59	29.41	72	3150	-	-	-	-	-	-
6	1.42	69.06	7.0	3156	-	-	-	-	-	-	-	-	-	-
7	1.45	69.33	7.0	3350	-	-	-	-	35	1.09	1700	11.95	39.1	6923
8	1.59	67.84	7.3	3719	66.12	33.88	72	3225	-	-	-	-	-	-
9	1.44	68.46	7.2	3257	68.81	31.19	-	-	-	-	-	-	-	-
10	1.48	68.52	7.3	3126	70.52	29.48	120	3050	-	-	-	-	-	-
11	1.45	67.68	7.1	3397	71.48	28.52	-	-	-	-	-	-	-	-
12	1.49	68.23	7.2	3250	-	-	60	2000	-	-	-	-	-	-
13	1.48	67.66	7.0	3136	77.10	22.90	-	-	-	-	-	-	-	-
14	1.45	67.71	7.0	3428	-	-	-	-	259	0.46	1875	9.58	50.3	7301
15	1.48	68.52	7.2	3562	-	-	312	2850	-	-	-	-	-	-
16	1.46	68.43	7.1	3403	72.08	27.92	-	-	-	-	-	-	-	-
17	1.47	68.67	7.2	3158	71.26	28.74	96	3025	-	-	-	-	-	-
18	1.56	69.03	7.1	3392	72.48	27.52	-	-	-	-	-	-	-	-
19	1.50	67.77	7.1	3209	71.27	28.73	96	2350	-	-	-	-	-	-
20	1.47	68.30	7.0	2990	-	-	-	-	-	-	-	-	-	-
21	1.59	70.14	7.0	3312	-	-	-	-	892	0.32	2025	5.69	34.2	7210
22	1.43	69.35	7.2	3283	70.08	29.92	48	2400	-	-	-	-	-	-
23	1.54	69.63	7.2	3524	70.40	29.60	-	-	-	-	-	-	-	-
24	1.47	68.36	7.2	3001	71.57	28.43	72	2450	-	2.70	-	-	-	-
25	1.49	69.05	7.1	3134	71.11	28.89	-	-	-	-	-	-	-	-
26	1.44	68.71	7.1	2971	72.25	27.75	72	1850	-	-	-	-	-	-
27	1.52	68.95	7.0	3161	-	-	-	-	-	-	-	-	-	-
28	1.48	69.50	7.1	2918	-	-	-	-	209	0.69	2075	6.71	21.8	8028
29	1.36	67.60	6.9	2876	70.61	29.39	96	2200	-	-	-	-	-	-
30	1.31	67.65	6.9	1831	70.48	29.52	-	-	-	-	-	-	-	-
31	1.33	67.46	7.0	2157	71.68	28.32	96	2450	-	-	-	-	-	-
32	1.37	68.48	6.9	2714	72.94	27.06	-	-	-	-	2200	-	-	-
33	1.34	66.94	7.0	2889	71.98	28.02	96	2300	-	-	-	-	-	-
34	1.53	68.30	6.9	4935	-	-	-	-	-	-	-	-	-	-
35	1.80	67.84	7.0	3077	68.18	31.82	96	2250	103	ND	2450	8.38	16.7	7762

* Filter yield.
† Specific resistance.